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CENTRE COLLABORATEUR OMS POUR LES SUBSTANCES CHIMIQUES DE REFERENCE

Rapport d'activité pour 1997

par M. Westernmark

Les substances chimiques internationales de référence nouvellement établies, proposées par le Centre collaborateur OMS pour les substances chimiques de référence sur la base d'essais et d'une caractérisation appropriés, figurent dans le rapport annuel du Centre. Ce rapport est communiqué, entre autres, aux membres du Tableau consultatif d'experts de la *Pharmacopée internationale* et des Préparations pharmaceutiques, auxquels il est demandé d'examiner soigneusement les propositions ainsi que la documentation jointe concernant les analyses effectuées, et de faire part au Centre de toute réserve ou critique avant la fin septembre 1998. En de tels cas, le Centre procédera à toutes consultations ou analyses complémentaires nécessaires pour la validation de la substance.

Si aucun commentaire négatif n'est reçu dans les trois mois, les nouvelles substances chimiques internationales de référence proposées peuvent être considérées comme *provisoirement* adoptées. Leur adoption *définitive* fera l'objet d'un examen au cours de la réunion suivante du Comité d'experts.

Prière d'adresser vos remarques à Mme M. Westernmark, Centre collaborateur OMS pour les substances chimiques de référence, Apoteket AB, Produktion & Laboratorier, Centrallaboratoriet (ACL), Prismavägen 2, S-14175 Kungens Kurva, Suède.

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Note : Pour des raisons techniques, les appendices 9 à 17 n'ont été établis qu'en anglais.

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Distribution de substances de référence en 1997

En 1997, le Centre a distribué 1559 substances chimiques internationales de référence. Ce chiffre représente une augmentation d'environ 34 % par rapport à celui de 1996. Les substances les plus fréquemment demandées ont été, dans l'ordre, l'acétate de vitamine A (acétate de rétinol), la phénacétine P.F., la vanilline P.F., la caféine P.F. et l'acide folique. On trouvera à l'appendice 1 le détail de la distribution des diverses substances.

En 1997, les substances ont été distribuées dans 38 pays. On trouvera à l'appendice 2 le détail de la distribution par pays. En ce qui concerne la distribution par Région de l'OMS, on constate qu'environ 14,3 % des substances ont été fournies à la Région africaine, 3,7 % à la Région des Amériques, 14,4 % à la Région de la Méditerranée orientale, 60,4 % à la Région européenne, 2,9 % à la Région de l'Asie du Sud-Est, et 4,4 % à la Région du Pacifique occidental.

Distribution de spectres de référence en 1997

Aucun spectre de référence n'a été distribué en 1997.

Etablissement de substances de référence en 1997

Conformément à la procédure recommandée par le Comité OMS d'experts des Spécifications relatives aux Préparations pharmaceutiques dans son trente-deuxième rapport (Série de Rapports techniques, N° 823), huit substances chimiques internationales de référence ont été établies en 1997. On trouvera la liste de ces substances à l'appendice 3.

On trouvera à l'appendice 4 une liste complète de toutes les substances chimiques internationales de référence détenues par le Centre en janvier 1998, avec indication de la quantité de substance contenue dans chaque unité de conditionnement et du numéro de contrôle des lots actuels. Cette liste comprend également neuf substances mentionnées ci-dessous, dont on peut prévoir qu'elles seront officiellement adoptées par le Comité d'experts à sa prochaine réunion.

Etablissement de spectres de référence en 1997

On trouvera à l'appendice 5 une liste complète de tous les spectres infrarouges internationaux de référence avec indication des nouveaux spectres établis depuis 1993. Aucun nouveau spectre n'a été établi en 1997.

Travaux effectués en 1997 sur de nouvelles substances de référence

Le Centre a poursuivi ses travaux en vue de fournir de nouvelles substances de référence qui seront nécessaires pour accompagner les spécifications de la troisième édition de la *Pharmacopée internationale*. En 1997, sept nouvelles substances de référence destinées à accompagner les volumes 3, 4 et 5 ont été examinées : captopril, chlorhydrate de ciprofloxacine, cisplatine, monosulfate de kanamycine, adipate de pipérazine, citrate de pipérazine et sulfate de streptomycine. Les rapports d'analyse pour ces substances figurent aux appendices 10-13 et 14-17. Ces substances sont jugées satisfaisantes en vue de leur adoption comme substances chimiques internationales de référence. Les stocks N° 192171 d'acétanilide et N° 192172 de phénacétine étaient en cours d'épuisement et ont été remplacés par les stocks N° 297171 d'acétanilide et N° 297172 de phénacétine. On trouvera aux appendices 9 et 14 les rapports d'analyse de ces substances.

Essais de stabilité

Le Centre a poursuivi ses examens périodiques de la stabilité des substances chimiques internationales de référence existantes. En 1997, 33 substances ont été réexaminées. On trouvera les résultats de ce réexamen à l'appendice 6. On peut obtenir auprès du Centre des détails concernant les méthodes utilisées.

Travaux en cours et travaux futurs

Les travaux sur les substances nécessaires pour accompagner les monographies des volumes 3, 4 et 5 de la *Pharmacopée internationale* se poursuivent. Actuellement, le Centre procède à l'étude de 14 des 59 substances énumérées à l'appendice 7.

En 1997, le Centre a poursuivi ses activités de contrôle de la qualité. Un "contrôle des modifications" a, par exemple, été mis en place. Des modifications ont été apportées à la documentation qui accompagne les envois de substances de référence. Par exemple, depuis juillet 1997 tous les nouveaux certificats sont signés.

Trois boursiers sont venus au Centre en janvier et octobre : le Professeur Kamel Bouzouita et Hassen Trabelsi, du Laboratoire national de Contrôle des Médicaments, Tunisie, et Vasenta Perera, du National Drug Quality Assurance Laboratory, Sri Lanka. Ces boursiers ont étudié les aspects administratifs de la manutention des substances de référence ainsi que différentes techniques d'analyse utilisées dans l'essai des substances chimiques internationales de référence.

Questions administratives et financières

Le coût de fonctionnement total du Centre en 1997 a été estimé à US \$574 447. Le revenu de la vente de substances de référence a été d'environ US \$95 990. Le Siège de l'OMS a apporté une contribution de US \$16 000. Cela laisse un déficit de US \$462 457, couvert grâce au soutien d'Apoteket AB.

En 1997, le prix des substances a été maintenu à US \$60 par paquet, et les frais d'expédition et de manutention s'ajoutant à chaque commande ont été maintenus à US \$10.

Remerciements

Le Centre remercie les laboratoires qui ont contribué à ses travaux en 1997, notamment le Laboratoire de la Pharmacopée européenne, Strasbourg, France, l'Institute of Science and Forensic Medicine, Singapour, et le National Institute for Biological Standards and Control, Londres.

Le Centre désire également remercier les laboratoires pharmaceutiques qui lui ont fourni des substances pour examen et ont participé aux travaux d'analyse. Cette année, ses remerciements vont en particulier à Bayer, Leverkusen, Allemagne; Bristol-Myers Squibb, New Brunswick, Etats-Unis d'Amérique; et Heraeus GmbH, Hanau, Allemagne.

DISTRIBUTION DE SUBSTANCES CHIMIQUES DE REFERENCE EN 1997

SCIR	Echantillons vendus
Acéclidine, salicylate	1
<i>p</i> -Acétamidobenzalazine	3
Acétazolamide	5
Allopurinol	2
Amidotrizoïque, acide	0
2-Amino-5-nitrothiazole	1
3-Aminopyrazole-4-carboxamide, hémisulfate	3
3-Amino-2,4,6-triiodobenzoïque, acide	0
Amitriptyline, chlorhydrate	6
Amodiaquine, chlorhydrate	2
Amphotéricine B	4
Ampicilline (anhydre)	10
Ampicilline sodique	13
Ampicilline, trihydrate	24
Anhydrotétracycline, chlorhydrate	16
Atropine, sulfate	4
Azathioprine	6
Bacitracine zinc	3
Béclométasone, dipropionate	2
Bendazol, chlorhydrate	2
Benzobarbital	2
Benzylamine, sulfate	3
Benzylpénicilline potassique	31
Benzylpénicilline sodique	42
Béphénium, hydroxynaphtoate	2
Bétaméthasone	4
Bétaméthasone, phosphate sodique	2
Bétaméthasone, valérate	3
Bétanidine, sulfate	2
<i>NN'</i> -bis(2,3-xylyl)anthranilamide	2
Bupivacaïne, chlorhydrate	2
Caféine	5
Calcium, folinate (Leucovorine calcique)	1
Carbamazépine	6
Carbénicilline monosodique	2
Chloramphénicol	14
Chloramphénicol, palmitate	2
Chloramphénicol, palmitate (forme A)	3
5-Chloro-2-méthylaminobenzophénone	3
Chloroquine, sulfate	2
2-(4-Chloro-3-sulfamoylbenzoyl)benzoïque, acide	3
Chlorphénamine, hydrogénomaléate	4
Chlorpromazine, chlorhydrate	5
Chlortalidone	2
Chlortétracycline, chlorhydrate	26

SCIR

Echantillons vendus

Cimétidine	8
Clomifène, citrate	2
Clomifène, citrate (isomère Z) (<i>voir</i> Zuclofène)	
Cloxacilline sodique	19
Colécalciférol	4
Cortisone, acétate	1
Dapsone	1
Désoxycortone, acétate	2
Dexaméthasone	5
Dexaméthasone, acétate	3
Dexaméthasone - acide phosphorique	2
Dexaméthasone, phosphate sodique	7
Diazépam	4
Diazoxide	2
Dicloxacilline sodique	2
Dicolinium, iodure	2
Dicoumarol	2
Diéthylcarbamazine, dihydrogénocitrate	2
Digitoxine	9
Digoxine	10
Dopamine, chlorhydrate	2
Doxorubicine, chlorhydrate	0
Emétine, chlorhydrate	2
4-Epianhydrotétracycline, chlorhydrate	17
4-Epitétracycline, chlorhydrate	18
Ergocalciférol	26
Ergométrine, hydrogénomaléate	3
Ergotamine, tartrate	2
Erythromycine	2
Erythromycine B	0
Erythromycine C	0
Estradiol, benzoate	2
Estrone	2
Etacrynique, acide	2
Ethambutol, chlorhydrate	3
Ethinylestradiol	4
Ethistérone	2
Ethosuximide	2
Etocarlide	2
Flucloxacilline sodique	2
Flucytosine	2
Fludrocortisone, acétate	0
Fluorouracil	2
Fluphénazine, décanoate (dichlorhydrate)	2
Fluphénazine, énantate (dichlorhydrate)	2
Fluphénazine, chlorhydrate	2
Folique, acide	47
3-Formylrifamycine	8

SCIR

Echantillons vendus

Framycétine, sulfate (Néomycine B, sulfate)	8
Furosémide	7
Gentamicine, sulfate	10
Griséofulvine	3
Halopéridol	4
Hydrochlorothiazide	3
Hydrocortisone	9
Hydrocortisone, acétate	2
Hydrocortisone, succinate sodique	2
(-)-3-(4-Hydroxy-3-méthoxyphényl)-2-hydrazino-2-méthylalanine	2
(-)-3-(4-Hydroxy-3-méthoxyphényl)-2-méthylalanine	2
Ibuprofène	8
Imipramine, chlorhydrate	5
Indométacine	4
o-Iodohippurique, acide	2
Isoniazide	12
Lanatoside C	2
Lévodopa	4
Lévonorgestrel	1
Lévothyroxine sodique (Thyroxine sodique)	5
Lidocaïne	3
Lidocaïne, chlorhydrate	15
Liothyronine sodique	3
Lopéramide, chlorhydrate	1
Mébendazole	8
Méfénamique, acide	3
Métazide	2
Méthaqualone	2
Méthotrexate	2
Méthylropa	10
Méthyltestostérone	2
Méticilline sodique	30
Métronidazole	16
Nafcilline sodique	4
Néamine, chlorhydrate	2
Néomycine B, sulfate (<i>voir</i> Framycétine, sulfate)	
Néostigmine, méthilsulfate	4
Nicotinamide	4
Nicotinique, acide	3
Nifurtimox	0
Niridazole	2
Niridazole-chloréthylcarboxamide	2
Noréthistérone	4
Noréthistérone, acétate	2
Nystatine	43
Ouabaïne	2
Oxacilline sodique	3
Oxytétracycline, chlorhydrate	9

SCIR

Echantillons vendus

Oxytétracycline, dihydrate	11
Papavérine, chlorhydrate	2
Paracétamol	13
Paromomycine, sulfate	0
Phénéticilline potassique	2
Phénoxyméthylpénicilline	2
Phénoxyméthylpénicilline calcique	2
Phénoxyméthylpénicilline potassique	11
Phénytoïne	5
Praziquantel	1
Prednisolone	14
Prednisolone, acétate	2
Prednisolone, hémisuccinate	0
Prednisolone, phosphate sodique	24
Prednisone	3
Prednisone, acétate	3
Probenécide	2
Procaïne, chlorhydrate	3
Procarbazine, chlorhydrate	2
Progestérone	2
Propicilline potassique	2
Propranolol, chlorhydrate	7
Propylthiouracile	2
Pyrantel, embonate	5
Pyridostigmine, bromure	2
Réserpine	4
Rétinol, acétate (solution à 25 000 UI)	87
Riboflavine	7
Rifampicine	7
Rifampicine quinone	7
Sodium, cromoglicate	3
Spectinomycine, chlorhydrate	2
<i>Substances de référence pour le point de fusion</i>	
Azobenzène	26
Vanilline	70
Benzile	20
Acétanilide	30
Phénacétine	71
Benzanilide	26
Sulfanilamide	19
Sulfapyridine	17
Dicyandiamide	15
Saccharine	14
Caféine	70
Phénolphtaléine	27
Sulfacétamide	0
Sulfaméthoxazole	10
Sulfaméthoxypyridazine	4

SCIR

Echantillons vendus

Sulfanilamide	4
Sulfasalazine	2
Tamoxifène, citrate	1
Tamoxifène, citrate (isomère E)	0
Testostérone, énantate	0
Testostérone, propionate	1
Tétracycline, chlorhydrate	43
Thioacétazone	14
4,4'-Thiodianiline	2
Thyroxine sodique (<i>voir</i> Lévothyroxine sodique)	
Tolbutamide	2
Tolnaftate	2
Toluène-2-sulfonamide	0
Triméthadione	2
Triméthoprim	11
Triméthylguanidine, sulfate	2
Tubocurarine, chlorure	2
Vincristine, sulfate	7
Vitamine A, acétate (solution) (<i>voir</i> Rétinol, acétate)	
Warfarine	2
Zuclomifène	3

DISTRIBUTION DE SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DANS LES DIFFERENTES REGIONS DE L'OMS EN 1997

Régions OMS	Nombre de SCIR distribuées en 1997
Région africaine (AFRO)	
Ethiopie	170
Ouganda	28
Sierra Leone	22
Région des Amériques (AMRO)	
Argentine	10
Brésil	7
Cuba	8
Etats-Unis d'Amérique	29
Mexique	3
Région de la Méditerranée orientale (EMRO)	
Chypre	4
Libye	14
République arabe syrienne	20
Région européenne (EURO)	
Allemagne	266
Autriche	17
Belgique	16
Danemark	18
Espagne	12
Finlande	13
France	69
Hongrie	3
Irlande	6
Israël	6
Italie	5
Kirghizistan	181
Liechtenstein	3
Norvège	17
Pays-Bas	7
Pologne	15
Royaume-Uni	92
Slovaquie	2
Suède	145
Suisse	37
Région de l'Asie du Sud-Est (SEARO)	
Corée	3
Indonésie	3
Sri Lanka	39
Région du Pacifique occidental (WPRO)	
Laos	4
Philippines	26
Singapour	35
Taiwan	2

**SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE
ETABLIES EN 1997**

Substance de référence	N° de contrôle	Rapport d'analyse	Remarques
Amidotrizoïque, acide	196205	WHO/PHARM/96.587 Appendice 9	
3-Amino-2,4,6-triiodobenzoïque, acide	196206	WHO/PHARM/96.587 Appendice 10	
Bétaméthasone, phosphate sodique	196203	WHO/PHARM/96.587 Appendice 11	
Doxorubicine, chlorhydrate	196202	WHO/PHARM/96.587 Appendice 12	
Sulfacétamide	196200	WHO/PHARM/96.587 Appendice 13	
Tamoxifène, citrate	196208	WHO/PHARM/96.587 Appendice 14	
Tamoxifène, citrate (isomère E)	196209	WHO/PHARM/96.587 Appendice 15	
Toluène-2-sulfonamide	196204	WHO/PHARM/96.587 Appendice 16	

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DISPONIBLES

1998

Informations générales

Les substances chimiques internationales de référence sont établies conformément à l'avis du Comité OMS d'experts des Spécifications relatives aux Préparations pharmaceutiques. Elles sont fournies principalement pour être utilisées dans des épreuves physiques et chimiques ainsi que dans des dosages décrits dans les spécifications pour le contrôle de la qualité des produits pharmaceutiques publiées dans la *Pharmacopée internationale* ou proposées sous forme de projets de monographies. Les substances chimiques internationales de référence sont principalement destinées à servir d'étalons primaires pour l'étalonnage d'étalons secondaires.

Le mode d'emploi et les données analytiques pour l'usage auquel elles sont destinées dans la spécification correspondante de la *Pharmacopée internationale* sont fournis dans les certificats joints aux substances distribuées.

Les substances chimiques internationales de référence peuvent être utilisées également dans des épreuves et des dosages qui ne sont pas décrits dans la *Pharmacopée internationale*. Cependant, dans ce cas, il incombe à l'utilisateur ou à la Commission de la Pharmacopée, ou à toute autre autorité qui a prescrit l'utilisation de ces substances, de vérifier qu'elles conviennent à l'usage qui en est fait.

Il est en général recommandé de conserver les substances à l'abri de la lumière et de l'humidité et de préférence à une température voisine de +5°C. Lorsque des conditions spéciales de stockage sont nécessaires, l'indication en est portée sur l'étiquette ou figure dans la notice jointe aux substances. Il est recommandé à l'utilisateur de n'acheter que la quantité suffisante pour l'usage immédiat.

La stabilité des substances chimiques internationales de référence conservées au Centre est surveillée par des examens réguliers et, lorsque cela est nécessaire, les substances détériorées sont remplacées par de nouveaux lots. Des listes indiquant les numéros de contrôle des lots en cours sont publiées dans les rapports annuels du Centre et peuvent être obtenues sur demande.

Commandes de substances

Les commandes de substances chimiques internationales de référence doivent être envoyées à :

Centre collaborateur OMS pour les substances chimiques de référence
Apoteket AB
Produktion & Laboratorier
Centrallaboratoriet, ACL
Prismavägen 2
S-14175 Kungens Kurva
Suède

(Télécopie : + 46 8 740 6040)

Les substances chimiques internationales de référence sont exclusivement fournies par paquets standard contenant la quantité indiquée sur la liste ci-après.

Substances de référence	Conditionnement	N° de contrôle
Acéclidine, salicylate	100 mg	172048
<i>p</i> -Acétamidobenzalazine	100 mg	290042
Acétazolamide	100 mg	186128
Allopurinol	100 mg	287049
Amidotrizoïque, acide	100 mg	196205
2-Amino-5-nitrothiazole	25 mg	186131
3-Aminopyrazole-4-carboxamide, hémisulfate	100 mg	172050
3-Amino-2,4,6-triiodobenzoïque, acide	100 mg	196206
Amitriptyline, chlorhydrate	100 mg	181101
Amodiaquine, chlorhydrate	200 mg	192160
Amphotéricine B	400 mg	191153
Ampicilline (anhydre)	200 mg	390001
Ampicilline sodique	200 mg	388002
Ampicilline, trihydrate	200 mg	274003
Anhydrotétracycline, chlorhydrate	25 mg	180096
Atropine, sulfate	100 mg	183111
Azathioprine	100 mg	172060
Bacitracine zinc	200 mg	192174
Béclométasone, dipropionate	200 mg	192175
Bendazol, chlorhydrate	100 mg	173066
Benzobarbital	100 mg	172051
Benzylamine, sulfate	100 mg	172052
Benzylpénicilline potassique	200 mg	180099
Benzylpénicilline sodique	200 mg	280047
Béphénium, hydroxynaphtoate	100 mg	183112
Bétaméthasone	100 mg	183113
Bétaméthasone, phosphate sodique	100 mg	196203
Bétaméthasone, valérate	100 mg	190145
Bétanidine, sulfate	100 mg	172053
<i>NN'</i> -bis(2,3-xylyl)anthranilamide	50 mg	173067
Bupivacaïne, chlorhydrate	100 mg	289054
Caféine	100 mg	181102
Calcium, folinate (Leucovorine calcique)	100 mg	194188
Captopril	100 mg	197214
Carbamazépine	100 mg	189143
Carbénicilline monosodique	200 mg	383043
Chloramphénicol	200 mg	486004
Chloramphénicol, palmitate	1 g	286072
Chloramphénicol, palmitate (forme A)	200 mg	175073
5-Chloro-2-méthylaminobenzophénone	100 mg	172061
Chloroquine, sulfate	200 mg	195201
2-(4-Chloro-3-sulfamoylbenzoyl)benzoïque, acide	50 mg	181106
Chlorphénamine, hydrogénomaléate	100 mg	182109
Chlorpromazine, chlorhydrate	100 mg	178080
Chlortalidone	100 mg	183114
Chlortétracycline, chlorhydrate	200 mg	187138
Cimétidine	100 mg	190150
Ciprofloxacine, chlorhydrate	400 mg	197210
Cisplatine	100 mg	197207
Clomifène, citrate	100 mg	187136
Clomifène, citrate (isomère Z) (voir Zuclomifène)		
Cloxacilline sodique	200 mg	274005

Substances de référence	Conditionnement	N° de contrôle
Colécalciférol (Vitamine D ₃)	500 mg	190146
Cortisone, acétate	100 mg	167006
Dapsone	100 mg	183115
Désoxycortone, acétate	100 mg	167007
Dexaméthasone	100 mg	388008
Dexaméthasone, acétate	100 mg	288009
Dexaméthasone - acide phosphorique	100 mg	192161
Dexaméthasone, phosphate sodique	100 mg	192158
Diazépam	100 mg	172062
Diazoxide	100 mg	181103
Dicloxacilline sodique	200 mg	174071
Dicolinium, iodure	100 mg	172055
Dicoumarol	100 mg	178077
Diéthylcarbamazine, dihydrogénocitrate	100 mg	181100
Digitoxine	100 mg	277010
Digoxine	100 mg	587011
Dopamine, chlorhydrate	100 mg	192159
Doxorubicine, chlorhydrate	100 mg	196202
Emétine, chlorhydrate	100 mg	187134
4-Épianhydrotétracycline, chlorhydrate	25 mg	288097
4-Epitétracycline, chlorhydrate	25 mg	293098
Ergocalciférol (Vitamine D ₂)	500 mg	190147
Ergométrine, hydrogénomaléate	50 mg	277012
Ergotamine, tartrate	50 mg	385013
Erythromycine	250 mg	191154
Erythromycine B	150 mg	194186
Erythromycine C	25 mg	194187
Estradiol, benzoate	100 mg	167014
Estrone	100 mg	279015
Etacrynique, acide	100 mg	281056
Ethambutol, chlorhydrate	100 mg	179081
Ethinylestradiol	100 mg	291016
Ethistérone	100 mg	167017
Ethosuximide	100 mg	179088
Etocarlide	100 mg	172057
Flucloxacilline sodique	200 mg	195194
Flucytosine	100 mg	184121
Fludrocortisone, acétate	200 mg	195199
Fluorouracil	100 mg	184122
Fluphénazine, chlorhydrate	100 mg	176076
Fluphénazine, décanoate (dichlorhydrate)	100 mg	182107
Fluphénazine, énantate (dichlorhydrate)	100 mg	182108
Folique, acide	100 mg	388019
3-Formylrifamycine	200 mg	190149
Framycétine, sulfate (Néomycine B, sulfate)	200 mg	193178
Furosémide	100 mg	171044
Gentamicine, sulfate	100 mg	194183
Griséofulvine	200 mg	280040
Halopéridol	100 mg	172063
Hydrochlorothiazide	100 mg	179087
Hydrocortisone	100 mg	283020
Hydrocortisone, acétate	100 mg	280021

Substances de référence	Conditionnement	N° de contrôle
Hydrocortisone, succinate sodique	200 mg	194184
(-)-3-(4-Hydroxy-3-méthoxyphényl)-2-hydrazino- 2-méthylalanine (3-O-Méthylcarbidopa)	25 mg	193180
(-)-3-(4-Hydroxy-3-méthoxyphényl)-2-méthylalanine (3-O-Méthylmétyldopa)	25 mg	179085
Ibuprofène	100 mg	183117
Imipramine, chlorhydrate	100 mg	172064
Indométacine	100 mg	178078
o-Iodohippurique, acide	100 mg	171045
Isoniazide	100 mg	185124
Kanamycine, monosulfate	12 mg	197211
Lanatoside C	100 mg	281022
Lévodopa	100 mg	295065
Lévonorgestrel	200 mg	194182
Lévothyroxine sodique	100 mg	189144
Lidocaïne	100 mg	181104
Lidocaïne, chlorhydrate	100 mg	181105
Liothyronine sodique	50 mg	193179
Lopéramide, chlorhydrate	100 mg	194185
Mébendazole	200 mg	195195
Méfénamique, acide	100 mg	173068
Métazide	100 mg	172058
Méthaqualone	100 mg	173069
Méthotrexate	100 mg	194193
Méthylidopa	100 mg	179084
Méthyltestostérone	100 mg	167023
Méticilline sodique	200 mg	274024
Métronidazole	100 mg	183118
Nafcilline sodique	200 mg	272025
Néamine, chlorhydrate (Néomycine A, chlorhydrate)	0,5 mg	193177
Néomycine B, sulfate (<i>voir</i> Framycétine, sulfate)		
Néostigmine, métilsulfate	100 mg	187135
Nicotinamide	100 mg	179090
Nicotinique, acide	100 mg	179091
Nifurtimox	100 mg	194189
Niridazole	200 mg	186129
Niridazole-chloréthylcarboxamide	25 mg	186130
Noréthistérone	100 mg	186132
Noréthistérone, acétate	100 mg	185123
Nystatine	200 mg	191152
Ouabaïne	100 mg	283026
Oxacilline sodique	200 mg	382027
Oxytétracycline, chlorhydrate	200 mg	189141
Oxytétracycline, dihydrate	200 mg	189142
Papavérine, chlorhydrate	100 mg	185127
Paracétamol	100 mg	195198
Paromomycine, sulfate	75 mg	195197
Phénéticilline potassique	200 mg	167028
Phénoxy méthylpénicilline	200 mg	179082
Phénoxy méthylpénicilline calcique	200 mg	179083
Phénoxy méthylpénicilline potassique	200 mg	176075
Phénytoïne	100 mg	179089

Substances de référence	Conditionnement	N° de contrôle
Pipérazine, adipate	100 mg	197212
Pipérazine, citrate	100 mg	197213
Praziquantel	100 mg	194191
Prednisolone	100 mg	389029
Prednisolone, acétate	100 mg	289030
Prednisolone, hémisuccinate	200 mg	195196
Prednisolone, phosphate sodique	200 mg	194190
Prednisone	100 mg	167031
Prednisone, acétate	100 mg	169032
Probénécide	100 mg	192156
Procaïne, chlorhydrate	100 mg	183119
Procarbazine, chlorhydrate	100 mg	184120
Progestérone	100 mg	167033
Propicilline potassique	200 mg	274034
Propranolol, chlorhydrate	100 mg	187139
Propylthiouracile	100 mg	185126
Pyrantel, embonate	500 mg	192157
Pyridostigmine, bromure	100 mg	182110
Réserpine	100 mg	186133
Rétinol, acétate (solution)	5 capsules*	791038
Riboflavine	250 mg	382035
Rifampicine	200 mg	191151
Rifampicine quinone	200 mg	190148
Sodium, cromoglicate	100 mg	188140
Spectinomycine, chlorhydrate	200 mg	193176
Streptomycine, sulfate	100 mg	197215
<i>Substances de référence pour le point de fusion</i>		
Azobenzène (69°C)	4 g	192168
Vanilline (83°C)	4 g	192169
Benzile (96°C)	4 g	294170
Acétanilide (116°C)	4 g	297171
Phénacétine (136°C)	4 g	297172
Benzanilide (165°C)	4 g	192173
Sulfanilamide (166°C)	4 g	192162
Sulfapyridine (193°C)	4 g	192163
Dicyandiamide (210°C)	4 g	192164
Saccharine (229°C)	4 g	192165
Caféine (237°C)	4 g	192166
Phénolphtaléine (263°C)	4 g	192167
Sulfacétamide	100 mg	196200
Sulfaméthoxazole	100 mg	179092
Sulfaméthoxypyridazine	100 mg	178079
Sulfanilamide	100 mg	179094
Sulfasalazine	100 mg	191155
Tamoxifène, citrate	100 mg	196208
Tamoxifène, citrate (isomère E)	10 mg	196209
Testostérone, énantate	200 mg	194192
Testostérone, propionate	100 mg	167036
Tétracycline, chlorhydrate	200 mg	180095
Thioacétazone	100 mg	171046
4,4'-Thiodianiline	50 mg	183116
Thyroxine sodique (voir Lévothyroxine sodique)		

Substances de référence	Conditionnement	N° de contrôle
Tolbutamide	100 mg	179086
Tolnaftate	100 mg	176074
Toluène-2-sulfonamide	100 mg	196204
Triméthadione	200 mg	185125
Triméthoprim	100 mg	179093
Triméthylguanidine, sulfate	100 mg	172059
Tubocurarine, chlorure	100 mg	170037
Vitamine A, acétate (solution) (<i>voir</i> Rétinol, acétate)		
Vincristine, sulfate	9,7 mg/flacon	193181
Warfarine	100 mg	168041
Zuclomifène	50 mg	187137

* Par capsule, environ 9 mg dans 250 mg d'huile.



WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

LISTE DES SPECTRES INFRAROUGES INTERNATIONAUX DE REFERENCE DISPONIBLES

Le Centre collaborateur OMS pour les substances chimiques de référence fournit également 69 spectres infrarouges internationaux de référence.

Les prix sont actuellement de US \$5 par spectre et US \$200 par série de 50 spectres au choix (voir liste ci-dessous) sous classeur cartonné. Le classeur peut être commandé séparément pour US \$10.

Des frais administratifs de US \$10 couvrant la manutention et l'expédition par avion (lettre ou paquet-poste) sont ajoutés à chaque commande.

Les commandes doivent être envoyées à :

Centre collaborateur OMS pour les substances chimiques de référence
Apoteket AB
Produktion & Laboratorier
Centrallaboratoriet, ACL
Prismavägen 2
S-14175 Kungens Kurva
Suède

Télex : 11553 APOBOL S
Télécopie : + 46 8 740 6040.

Le paiement doit être effectué dans le délai indiqué sur la facture. Veuillez effectuer le paiement, en indiquant le numéro de facture, auprès de :

Postgirot Utland
S-10506 Stockholm
Suède

a/c N° : 29840-6, Apoteksbolaget AB/ACL,
SWIFT : PGSI SE SS

Les spectres infrarouges internationaux de référence suivants sont actuellement disponibles auprès du Centre :

acéclidine, salicylate
acétazolamide
allopurinol
amiloride, chlorhydrate
amitriptyline, chlorhydrate
ampicilline, trihydrate

béclométasone, dipropionate
benzylpénicilline potassique
bipéridène
bipéridène, chlorhydrate
bupivacaïne, chlorhydrate

caféine (anhydre)
calcium, folinate
carbidopa
chlorphénamine, hydrogénomaléate
clofazimine
cloxacilline sodique
colchicine
cytarabine

dexaméthasone
dexaméthasone, acétate, monohydrate
dextrométhorphan, bromhydrate
diazépam
dicolinium, iodure
dicoumarol
diéthylcarbamazine, dihydrogénocitrate
diphénoxylate, chlorhydrate

érythromycine, éthylsuccinate
érythromycine, stéarate
étacrynique, acide
éthionamide
éthosuximide

furosémide

gallamine, triéthiodure
glibenclamide

halopéridol
hydrochlorothiazide

ibuprofène
imipramine, chlorhydrate
indométacine
isoniazide

lidocaïne
lidocaïne, chlorhydrate
lindane

métronidazole
miconazole, nitrate

niclosamide
nicotinamide
noscapine

oxamniquine

papavérine, chlorhydrate
phénobarbital
phénoxy méthylpénicilline calcique
phénytoïne
primaquine, phosphate
propylthiouracile
protionamide
pyriméthamine

salbutamol
salbutamol, sulfate
sulfadimidine
sulfadoxine
sulfaméthoxazole
sulfaméthoxypyridazine

tiabendazol
trihexyphénidyle, chlorhydrate
triméthoprim

valproïque, acide
vérapamil, chlorhydrate

ESSAIS DE STABILITE

La stabilité des substances chimiques internationales de référence pendant leur stockage est surveillée par un réexamen périodique des substances détenues par le Centre. Les résultats obtenus pour les substances réexaminées en 1997 sont résumés ci-après. A titre comparatif, on a aussi indiqué les résultats obtenus lors des réexamens précédents. Les substances ont été conservées dans des récipients étanches à +5°C et sous une humidité relative inférieure à 30 %. Dans les tableaux, on a adopté les abréviations suivantes :

CE	Electrophorèse en tube capillaire
DSC	Calorimétrie différentielle
DTA	Analyse thermique différentielle
GC	Chromatographie en phase gazeuse
HPLC	Chromatographie liquide à haute performance
IR	Spectrophotométrie infrarouge
KF	Méthode de Karl Fischer pour la détermination de la teneur en eau
LC-MS	Chromatographie en phase liquide/spectrométrie de masse
LOD	Perte à la dessiccation
RMN	Résonance magnétique nucléaire
PSA	Analyse de solubilité par phases
TLC	Chromatographie en couche mince
TGA	Analyse thermogravimétrique

La valeur estimée des impuretés solides totales, obtenue par HPLC, CE et TLC, est exprimée en aire %, sauf indication contraire; lorsqu'elle est obtenue par DSC et par DTA, elle est exprimée en mole %, et par PSA en poids %. Les pertes de poids, mesurées par LOD et TGA, sont exprimées en poids %. Les valeurs obtenues par titrage sont calculées par rapport à la substance desséchée ou anhydre, sauf indication contraire.

Pour plus de détails sur les méthodes d'analyse utilisées, on peut s'adresser au Centre.

Amodiaquine, chlorhydrate, N° de contrôle 192160

Premier rapport d'analyse : WHO/PHARM/93.564, appendice 7

Année d'examen :	1992	1997
IR	conforme	-
TLC, %	environ 0,6	-
HPLC, %	0,3	0,4
TGA, %	7,9	7,8
Eau (KF), %	8,0	-
Titrage (spectrophotométrique), %	99,9	-

Ampicilline, trihydrate, N° de contrôle 274003

Premier rapport d'analyse : WHO/PHARM/75.485, appendice 6

Année d'examen :	1974	1989	1992	1997
IR	conforme	-	-	conforme
HPLC, %	-	0,3	0,3	0,5*
TGA, %	-	13,9	13,8	13,7
Eau (KF), %	13,9	-	-	-
Titration (alcalimétrique), %	98,5	-	-	-
Titration (mercurimétrique), %	-	-	98,8	-

* Nouvelle colonne ayant une meilleure efficacité de séparation.

Atropine, sulfate, N° de contrôle 183111

Premier rapport d'analyse : WHO/PHARM/84.513, appendice 5

Année d'examen :	1983	1990	1997
IR	conforme	-	conforme*
TLC, %	3 taches secondaires faibles	<0,1	<0,1*
HPLC, %	0,6	0,5	0,5
TGA, %	-	3,2	3,7
Eau (KF), %	3,1	-	3,8
LOD, %	3,0	-	-
Titration (potentiométrique), %	100,4	-	-

* Le réexamen de cette substance a été en partie réalisé par le Department of Scientific Services, Institute of Science and Forensic Medicine, Singapour.

Azathioprine, N° de contrôle 172060

Premier rapport d'analyse : WHO/PHARM/72.471, appendice 11, WHO/PHARM/73.473, appendice 3

Année d'examen :	1973	1979	1981	1997
IR	conforme	-	-	conforme
TLC, %	environ 0,5	1 tache	1 tache	-
HPLC, %	-	-	-	0,5
TGA, %	-	-	-	0,6
LOD, %	0,7	0,7	-	-
PSA, %	environ 1	-	-	-

Benzobarbital, N° de contrôle 172051

Premier rapport d'analyse : WHO/PHARM/72.471, appendice 13

Année d'examen :	1972	1977	1981	1987	1997
IR	conforme	-	-	conforme	-
TLC, %	0 tache secondaire	1 tache secondaire	2 taches secondaires	environ 0,5	-
HPLC, %	-	-	-	0,3	0,7
TGA, %	-	-	-	-	0,1
Eau (KF), %	-	-	-	0,1	-
LOD, %	0,4	<0,1	0,1	-	-
DTA, %	-	-	-	environ 1	-
Titrage (titrimétrique), %	100,0	100,2	-	100,3	-

Benzylamine, sulfate, N° de contrôle 172052

Premier rapport d'analyse : WHO/PHARM/72.471, appendice 14

Année d'examen :	1972	1977	1979	1997
IR	conforme	-	-	conforme
TLC, %	1 tache secondaire	1 tache secondaire		-
HPLC, %	-	-		<0,05
TGA, %	-	-	-	<0,1
LOD, %	<0,1	-	-	-
LC-MS	-	-	-	confirme l'identité

Carbamazépine, N° de contrôle 189143

Premier rapport d'analyse : WHO/PHARM/90.547, appendice 7

Année d'examen :	1989	1997
IR	conforme	-
TLC, %	<0,01	-
HPLC, %	<0,005	0,06*
TGA, %	<0,1	0,1
Titrage (spectrophotométrique), %	100,2	-

* Nouveau système chromatographique ayant une meilleure efficacité de séparation.

Chloramphénicol, N° de contrôle 486004

Premier rapport d'analyse : WHO/PHARM/87.532, appendice 8

Année d'examen :	1986	1992	1997
IR	conforme	-	conforme*
TLC, %	0,2	-	1 tache très faible*
HPLC, %	0,2	0,3	0,2 (0,3*)
TGA, %	-	<0,1	<0,1
LOD, %	0,05	-	-
Titration (spectrophotométrique), %	99,8	100,0	99,6*

* Le réexamen de cette substance a été en partie réalisé par le Department of Scientific Services, Institute of Science and Forensic Medicine, Singapour.

Chloramphénicol, palmitate, N° de contrôle 286072

Premier rapport d'analyse : WHO/PHARM/87.532, appendice 9

Année d'examen :	1986	1992	1997
IR	conforme, pas de forme A	conforme, pas de forme A	-
DSC, %	2,7	2,2	-
TLC, %	environ 2,7	-	-
HPLC, %	3,1	3,1	2,9
TGA, %	0,1	<0,1	<0,1
Eau (KF), %	0,2	<0,1	-
LOD, %	<0,1	-	-
Titration (spectrophotométrique), %	100,2	100,0	-

Chloramphénicol, palmitate (forme A), N° de contrôle 175073

Premier rapport d'analyse : WHO/PHARM/75.485, appendice 10

Année d'examen :	1974	1992	1997
IR	conforme	conforme	-
DSC, %	0,6	0,7	-
TLC, %	2 taches	-	-
HPLC, %	-	environ 2	1,6
TGA, %	-	<0,1	0,1
Eau (KF), %	-	<0,1	-
LOD, %	0,26	-	-
Titration (titrimétrique), %	100,9	-	-
Titration (spectrophotométrique), %	-	100,0	-

5-Chloro-2-méthylaminobenzophénone, N° de contrôle 172061

Premier rapport d'analyse : WHO/PHARM/73.475, appendice 4

Année d'examen :	1972	1985	1991	1997
IR	conforme	-	-	conforme
TLC, %	0 tache secondaire	0 tache secondaire	0 tache secondaire	-
HPLC, %	-	-	0,8	1,0
TGA, %	-	-	<0,1	-
LOD, %	0,1	-	-	-
Eau (KF), %	-	-	-	0,3
DTA, %	0,4	0,5	-	-
DSC, %	-	-	0,5	-

2-(4-Chloro-3-sulfamoylbenzoyl)benzoïque, acide, N° de contrôle 181106

Premier rapport d'analyse : WHO/PHARM/82.509, appendice 8

Année d'examen :	1981	1997
IR	conforme	conforme
TLC, %	2 taches secondaires	2 taches secondaires
HPLC, %	0,4	0,2 (> limite de dosage)
TGA, %	-	<0,1
LOD, %	0,2	-
Titrage (potentiométrique), %	98,9	-

Chlorpromazine, chlorhydrate, N° de contrôle 178080

Premier rapport d'analyse : WHO/PHARM/79.499, appendice 4

Année d'examen :	1978	1984	1990	1997
IR	conforme	-	-	-
TLC, %	<0,5	<0,5	environ 0,2	-
HPLC, %	-	-	-	0,06
TGA, %	-	-	<0,1	<0,1
LOD, %	0,1	<0,1	-	-
DTA, %	0,37	0,33	-	-
PSA, %	environ 0,7	-	-	-

Chlortalidone, N° de contrôle 183114

Premier rapport d'analyse : WHO/PHARM/84.513, appendice 9

Année d'examen :	1983	1997
IR	conforme	-
TLC, %	0,3	-
HPLC, %	0,5	0,8
HPLC, acide 2-(4-chloro-3-sulfamoyl-benzoyl) benzoïque, %	0,1	0,1
LOD, %	0,1	-
TGA, %	-	<0,1
PSA, %	0,9	-
Titration (potentiométrique), %	99,5	-

Clomifène, citrate, N° de contrôle 187136

Premier rapport d'analyse : WHO/PHARM/88.537, appendice 8

Année d'examen :	1987	1997
IR	conforme	-
TLC, %	0 tache secondaire	-
HPLC, %	0,3	0,9*
Isomère Z (HPLC), %	environ 35 (aire %)	32,6 (p/p %)**
Isomère Z (RMN), %	33	-
TGA, %	0,3	-
Eau (KF), %	-	0,7
Titration (potentiométrique), %	97,6	-

* Nouvelle méthode plus sélective. Optimisation de la longueur d'onde de 254 nm à 263 nm.

** Avec la SCIR 187137 comme étalon externe.

Clomifène, citrate (isomère Z), N° de contrôle 187137

Premier rapport d'analyse : WHO/PHARM/88.537, appendice 9

Année d'examen :	1987	1997
IR	conforme	-
TLC, %	<0,1	-
HPLC, %	0,3	0,3*
Isomère E (HPLC), %	environ 2 (aire %)	2,1 (p/p %)**
TGA, %	0,2	-
Eau (KF), %	-	0,2
Titration (potentiométrique), %	99,2	-

* Nouvelle méthode plus sélective. Optimisation de la longueur d'onde de 254 nm à 263 nm.

** Par rapport à un étalon externe.

Dapsone, N° de contrôle 183115

Premier rapport d'analyse : WHO/PHARM/85.513, appendice 10

Année d'examen :	1983	1988	1997
IR	conforme	-	-
TLC, %	2-4 taches	1	-
HPLC, %	1,2	1,9	0,7*
LC-MS (identification de l'impureté principale)			identifiée comme 4-aminodiphénylsulfone
PSA, %	1,0	-	-
TGA, %	-	<0,1	0,1
LOD, %	0,1	-	-
Titration (potentiométrique), %	99,9	100,0	-

* Nouvelle méthode HPLC. L'ancienne n'était pas linéaire.

Désoxycortone, acétate, N° de contrôle 167007

Premier rapport d'analyse : WHO/PHARM/66.431, appendice 1

Année d'examen :	1965	1975	1980	1984	1997
IR	conforme	-	conforme	-	-
TLC, %	aucune impureté détectée	aucune impureté détectée	-	2 taches détectées	-
HPLC, %	-	-	-	0,2	0,7*
TGA, %	-	-	-	-	<0,1
LOD, %	<0,1	0,1	-	<0,1	-
DTA, %	-	-	0,7	-	-
PSA, %	<1	-	-	-	-
Titration (spectrophotométrique), %	-	-	100	-	-

* Nouveau système de chromatographie en phase liquide ayant une meilleure efficacité de séparation.

Dexaméthasone – acide phosphorique, N° de contrôle 192161

Premier rapport d'analyse : WHO/PHARM/93.564, appendice 10

Année d'examen :	1992	1997
IR	conforme	-
TLC, %	0,1	-
HPLC, %	0,1	0,2
TGA, %	1	environ 1
Eau (KF), %	0,7	0,7
LOD, %	1,0	-
Titration (spectrophotométrique), %	100,2	-

Diazépam, N° de contrôle 172062

Premier rapport d'analyse : WHO/PHARM/73.475, appendice 5

Année d'examen :	1972	1982	1991	1997
IR	conforme	-	conforme	-
TLC, %	1 tache secondaire	1 tache secondaire	2 taches secondaires très faibles	-
HPLC, %	-	-	<0,05	<0,1
DTA, %	0,05	0,04	-	-
DSC, %	-	-	0,05	-
TGA, %	-	-	-	<0,1
LOD, %	<0,1	<0,1	-	-
Eau (KF), %	-	-	-	0,1
Titration (potentiométrique), %	100,0	100,0	-	-

Dicloxacilline sodique, N° de contrôle 174071

Premier rapport d'analyse : WHO/PHARM/74.478, appendice 5

Année d'examen :	1974	1982	1984	1989	1995	1997
IR	conforme	-	-	-	conforme	-
TLC, %	-	-	-	-	5 taches secondaires	-
HPLC, %	-	0,3	0,4	0,3	0,5	0,4
TGA, %	-	-	-	3,9	3,9	3,9
Eau (KF), %	3,8	3,9	3,8	-	-	-
Produits de dégradation (mercurimétrie), %	-	-	0,6	-	-	-
Titration (alcalimétrique), %	99,5	99,4	-	-	-	-
Titration (mercurimétrie), %	-	-	99,5	-	-	-

Dicoumarol, N° de contrôle 178077

Premier rapport d'analyse : WHO/PHARM/79.499, appendice 5

Année d'examen :	1978	1997
IR	conforme	-
TLC, %	0 tache secondaire	-
HPLC, %	<0,05	<0,04 (limite de détection)* <0,1 (limite de dosage)
TGA, %	-	<0,1
LOD, %	<0,1	-
Titration (potentiométrique), %	100,0	-
PSA, %	0,3	-

* Nouvelle méthode de chromatographie en phase liquide (passage des phases normales aux phases inversées). Limite de dosage 0,1 %, limite de détection 0,04 %.

Erythromycine, N° de contrôle 191154

Premier rapport d'analyse : WHO/PHARM/92.558, appendice 8

Année d'examen :	1991	1997
IR	conforme	-
TLC, %	environ 1	-
HPLC, %	environ 1,6	1,5
TGA, %	2,2	2,6
Eau (KF), %	-	1,4
Titration (titrimétrique), %	97,6	-
Activité, HPLC	962 µg/mg	-
Titration (microbiologique)	915 UI/mg	-

Hydrochlorothiazide, N° de contrôle 179087

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 8

Année d'examen :	1979	1987	1997
IR	conforme	conforme	-
DTA, %	0,4	0,7	-
HPLC, %	0,4	0,4	0,5
TGA, %	-	-	0,2
LOD, %	<0,1	0,2	-
Titrage (titrimétrique), %	100,0	-	-

Méthylidopa, N° de contrôle 179084

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 10

Année d'examen :	1979	1984	1992	1997
IR	conforme	-	-	-
TLC, %	0,2	-	0,1	-
HPLC, %	<0,2	0,2	0,1	0,1
TGA, %	-	-	11,7	11,4
Eau (KF), %	11,5	11,5	-	11,3
Titrage (potentiométrique), %	99,7	-	-	-

Méticilline sodique, N° de contrôle 274024

Premier rapport d'analyse : WHO/PHARM/74.478, appendice 9

Année d'examen :	1974	1978	1984	1997
IR	conforme	-	-	-
HPLC, %	-	<0,1	0,2	0,2
TGA, %	-	-	-	4,5
Eau (KF), %	4,7	-	4,6	-
Titration (alcalimétrique), %	99,9	-	-	-
Titration (mercurimétrique), %	-	-	99,5	-

Nafcilline sodique, N° de contrôle 272025

Premier rapport d'analyse : WHO/PHARM/72.471, appendice 6

Année d'examen :	1972	1978	1984	1997
IR	conforme	-	-	-
HPLC, %	-	<0,1	0,1	0,6*
TGA, %	-	-	-	4,7
Eau (KF), %	4,0	-	4,2	4,1
Composés organiques volatils, GC, %	-	-	-	0,6
Titration (mercurimétrique), %	-	-	99,1	-
Titration (alcalimétrique), %	99,9	-	-	-

* Nouveau système chromatographique avec détection à 225 nm.

Nystatine, N° de contrôle 191152

Premier rapport d'analyse : WHO/PHARM/92.558, appendice 10

Année d'examen :	1991	1992	1993	1994	1995	1996	1997
IR	conforme	-	-	-	-	-	-
HPLC, %, impuretés, 304 nm	5,9	-	7,9*	8,7	9,1	8,8 8,3****	8,5 (mars) 11,2 (octobre)
CE, %, impuretés, 304 nm	-	-	-	-	-	-	7 (octobre)
HPLC, titrage, %	100,0	-	100,0	100,5	≈99	≈97	-
TGA, %	5,0	-	5,2	5,3	5,1	4,9	5,1 (mars) 5,0 (octobre)
Eau (KF), %	4,8	-	-	-	5,3	5,1	-
Titration microbiologique, UI/mg	6 382**	5 208***	4 962***	4 868***	4 728***	4 690***	4 586*** (mars) 4 622*** (octobre)
TLC, %	4,1 (3 taches secondaires)	-	3 taches secondaires	-	-	-	-
UV, titrage, 304 nm, %	100,0	-	100,0	-	-	≈98	-

* Ces valeurs élevées sont dues à un nouveau système chromatographique ayant une meilleure efficacité de séparation.

** L'EPCRS, dont la teneur figurant sur l'étiquette est fautive, a été utilisée comme étalon.

*** Le 2^e étalon biologique OMS, dont la teneur déclarée est de 4855 UI/mg, a été utilisé comme étalon.

**** Nouvelle colonne HPLC.

Remarques concernant le réexamen d'octobre 1997 : une nouvelle méthode HPLC validée, donnant une meilleure sélectivité, a été adoptée. La tendance observée au niveau des impuretés détectées par HPLC ne traduit pas une dégradation, mais l'utilisation de méthodes d'analyse de plus en plus sélectives. L'intention était de changer de lot de nystatine, mais les autres substances examinées contenaient davantage d'impuretés. Par exemple, le 2^e étalon biologique OMS contient 16,8 % d'impuretés. La SCIR actuelle est donc la plus pure chimiquement. Le Centre continuera à rechercher des lots encore plus purs. Une méthode de CE a également été développée, mais la méthode HPLC a une sensibilité et une sélectivité supérieures.

En ce qui concerne le titrage microbiologique, un calcul a montré, que lorsqu'on comparait la valeur obtenue en 1995 (4728 UI/mg) à celles de 1996 et 1997, la différence n'était pas statistiquement significative au niveau de confiance 95 % avec un test *t* par séries non appariées.

Ouabaine, N° de contrôle 283026

Premier rapport d'analyse : WHO/PHARM/84.513, appendice 14

Année d'examen :	1983	1987	1997
IR	conforme	-	-
TLC, %	0,2	0,3	-
HPLC, %	0,4	0,7	0,6
TGA, %	-	-	19,9
LOD, %	20,0	19,9	-
Titration (colorimétrique), %	100,1	100,0	-

Oxacilline sodique, N° de contrôle 382027

Premier rapport d'analyse : WHO/PHARM/85.510, appendice 8

Année d'examen :	1982	1984	1987	1990	1997
IR	conforme	-	-	-	-
HPLC, %	1,1	1,0	1,3	0,7	1,4*
TGA, %	-	-	-	-	4,2
Eau (KF), %	4,3	4,5	4,1	4,2	-
PSA, %	0,9	-	-	-	-
Titration (mercurimétrique), %	-	99,4	99,4	98,9	-
GC, %	1,1	-	-	-	1,1

* Nouveau système de chromatographie en phase liquide ayant une meilleure efficacité de séparation.

Phénéticilline potassique, N° de contrôle 167028

Premier rapport d'analyse : WHO/PHARM/68.448, appendice 7

Année d'examen :	1967	1978	1982	1984	1997
IR	conforme	-	-	-	conforme
TLC, %	2 impuretés	-	-	-	-
HPLC, %	-	0,4	0,5	0,6	0,7
TGA, %	-	-	-	-	0,2
Eau (KF), %	0,5	-	0,3	0,4	-
PSA, %	56,5 isomère L	-	-	-	-
Titration (mercurimétrique), %	99,1	-	-	99,5	-

Phénytoïne, N° de contrôle 179089

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 13

Année d'examen :	1979	1985	1992	1997
IR	conforme	-	-	conforme*
HPLC, %	environ 0,02	-	environ 0,02	<0,04 (0,02*)
TGA, %	-	-	0,1	<0,1
Eau (KF), %	-	-	0,1	-
LOD, %	<0,1	0,17	-	-
DSC, %	-	-	<0,1	-
Titration (potentiométrique), %	100,1	-	-	-
Titration (chromatographie en phase liquide), %	-	-	-	100,0*

* Le réexamen de cette substance a été en partie réalisé par le Department of Scientific Services, Institute of Science and Forensic Medicine, Singapour.

Propicilline potassique, N° de contrôle 274034

Premier rapport d'analyse : WHO/PHARM/75.485, appendice 8

Année d'examen :	1974	1984	1987	1990	1997
IR	conforme	-	-	-	-
HPLC, isomère L, %	-	-	environ 60	environ 60	-
HPLC, %	-	0,8	0,5	0,7	0,7
TGA, %	-	-	0,3	0,3	0,4
Eau (KF), %	0,3	0,4	0,2	0,3	-
PSA, %	57 % isomère L	-	-	-	-
Titration (mercurimétrique), %	-	98,2	98,3	97,9	-
Produits de dégradation (mercurimétrie), %	-	-	0,8	0,8	-
Titration (alcalimétrique), %	99,5	-	-	-	-
Titration (iodométrique), %	98,3	-	-	-	-
Titration (potentiométrique), %	98,3	-	-	-	-

SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE

Liste prévisionnelle pour 1998

Les substances chimiques internationales de référence ci-après sont nécessaires pour accompagner les spécifications qui figurent dans la troisième édition de la *Pharmacopée internationale* :

Volume 3

Noroxymorphone, chlorhydrate
(impureté du chlorhydrate de naloxone)

Volume 4

Dactinomycine
Iohexol
Médroxyprogestérone, acétate (*)
Néomycine, sulfate (*)

Sodium, amidotrizoate (*)
Thiopental sodique
Vinblastine, sulfate (*)

Volume 5

Albendazole
Alecurnium, chlorure
Amoxicilline, trihydrate
Artéether
Artémether
Artémisinine
Artésunate
Aténolol
Aténolol pour validation des colonnes
Benznidazole
9,9'-Bisanthracène-10,10'-(9*H*, 9'*H*)-dione
Captopril, disulfure (*)
Céfadroxil, monohydrate
Chloramphénicol, succinate sodique
Ciclosporine
Ciclosporine U
Clindamycine, chlorhydrate
Clindamycine, phosphate
1-Cyclopropyl-1,4-dihydro-4-oxo-6-fluoro-7-
((2-aminoéthyl)amino)-3-quinoline
carboxylique, acide (dérivé de
l'éthylènediamine) (*)
1-Cyclopropyl-1,4-dihydro-4-oxo-6-
(1-pipérazinyl)-7-chloro-3-quinoline
carboxylique, acide (*)
1-Cyclopropyl-1,4-dihydro-4-oxo-7-
(1-pipérazinyl)-3-quinoline carboxylique,
acide (*)
Dacarbazine
Dacarbazine, substance apparentée A
Dacarbazine, substance apparentée B

Dihydroartémisinine
Diméthyl 2,6-diméthyl-4-(2-nitrophényl)
pyridine-3,5-dicarboxylate
Diméthyl 2,6-diméthyl-4-(2-nitrosophényl)
pyridine-3,5-dicarboxylate
1,2-Diphényléthylammonium-3-mercapto-2-
méthylpropanoate (*)
Dithranol
Doxycycline, hyclate (*)
Econazole, nitrate
6-Epidoxycycline, chlorhydrate
Erythromycine, éthylsuccinate
Erythromycine, stéarate
Etoposide
Fluoroquinolinique, acide (*)
1-Hydroxy-9-anthrone
Idoxuridine
Kétoconazole
Lévamisole, chlorhydrate
Lincomycine, chlorhydrate (*)
Méfloquine, chlorhydrate
Métacycline, chlorhydrate
DL-Méthionine
2-Méthyl-3-acétylthiopropionique, acide (*)
Métronidazole, benzoate
Nifédipine
Nonoxinol 9
Octoxinol 9
Rétinol, palmitate
Rétinol, propionate

(*) Indique que des travaux sont en cours au Centre sur cette substance.

SPECTRES INFRAROUGES INTERNATIONAUX DE REFERENCE**Liste prévisionnelle pour 1998**

Les spectres infrarouges internationaux de référence ci-après sont nécessaires pour accompagner les spécifications qui figurent dans la troisième édition de la *Pharmacopée internationale* :

Volume 3

Diloxanide, furoate
Métoclopramide, chlorhydrate (*)
Naloxone, chlorhydrate (*)
Nitrofurantoïne
Pyrazinamide (*)
Spironolactone (*)

Volume 4

Disodium, édetate
Ephédrine, sulfate
Iopanoïque, acide
Iotroxique, acide
Kétamine, chlorhydrate
Noréthistérone, énantate
Pentamidine, isétionate
Timolol, maléate

Volume 5

Azote, protoxyde
Benzoyle, peroxyde
Ciprofloxacine
Codéine, phosphate
Colchicine
Diéthyltoluamide
Diloxanide, furoate
Erythromycine, lactobionate
Isosorbide, dinitrate
Morphine, sulfate
Péthidine, chlorhydrate
Phénobarbital
Proguanil, chlorhydrate
Sulfadiazine
Tropicamide

(*) Travaux en cours.

Acetanilide

WHO Melting Point Reference Substance

Melting temperature 116 °C

Control No 297171

Analytical Report

Intended use

The stock of the current batch of the WHO Melting Point Reference Substance for acetanilide, Control No 192171, is depleted and has to be replaced.

The WHO Melting Point Reference Substance for acetanilide is supplied primarily for calibration of different instruments and methods used for determination of melting temperatures against the method of *The International Pharmacopoeia*, Third Edition, Volume 1.

Material

About 1000 g of the sample (manufacturers batch no K22532110) were received at the WHO Centre in May 1997. The material is being stored in tightly closed containers at + 20 °C, protected from light.

Analytical data

Description

A white crystalline powder.

Evidence of chemical structure

Infrared spectrum

The spectrum is concordant with the spectrum of the previous lot of the International Chemical Reference Substance (ICRS) of acetanilide with Control No 192171. An infrared spectrum is given in Figure 1 (No W297171T).

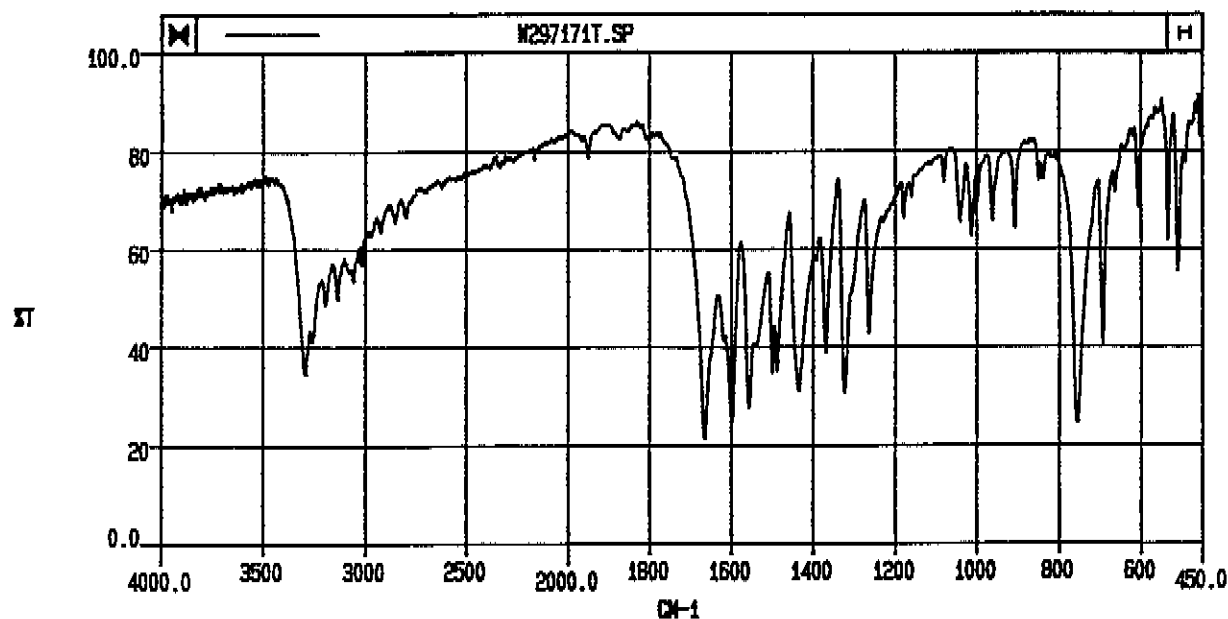


Figure 1. IR-spectrum of 1.0 mg of acetanilide Control No 297171 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

Purity

Assigned melting point: 116 °C

Based on results from a collaborative study. See under Collaborative study at the end of this Appendix.

Thermogravimetric analysis

The substance is already volatile at 90 °C, therefore thermogravimetric analysis is not a suitable method.

Water

< 0.1 % (n=2) determined by Karl Fischer titration.

High performance liquid chromatography

No impurities above the limit of quantification (0.08 %) were found. A chromatogram is given in Figure 2.

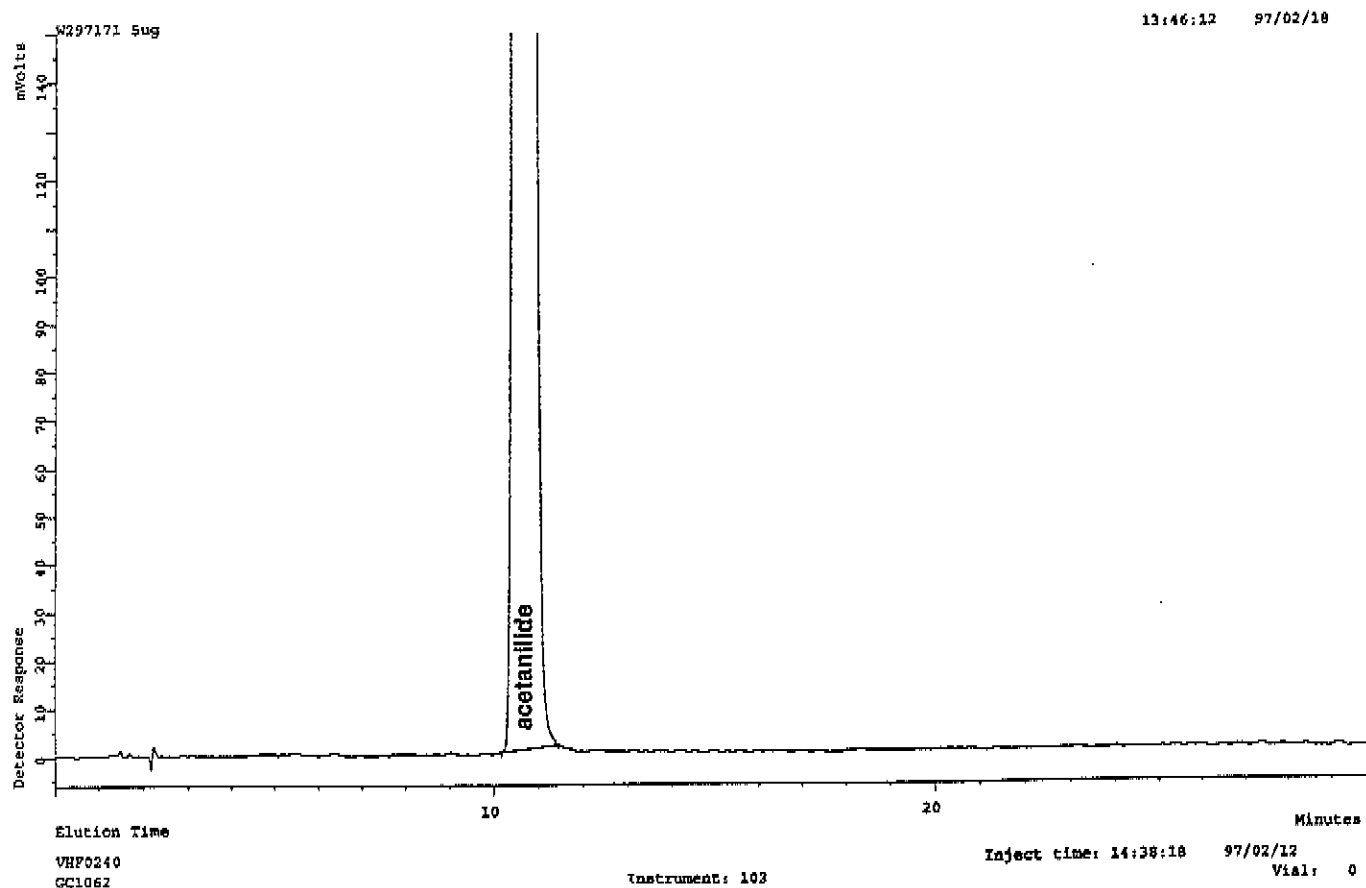


Figure 2. *Chromatogram of acetanilide Control No 297171 monitored at 240 nm.*

The following conditions were used:

Eluent:	Acetonitrile:0.05 M phosphate buffer pH 3.0 (15:85)
Column:	Genesis C18, 4 μ m, 4.6 x150 mm
Detector:	Gynkotek UVD 340S operated at 240 nm.
Pump:	Gynkotek M480 operated at a flow rate of 1.0 ml/min.
Injector:	Gynkotek Gina 50 operated at 8 °C.
Integrator:	PeakPro (Beckman)
Sample:	Acetanilide was dissolved in the eluent at a concentration of 1.0 mg/ml. 5 μ l corresponding to 5 μ g were injected.
Stability in the eluent:	A sample was stored in daylight for one week in the eluent. No signs of degradation were observed.
Limit of detection:	1 ng (0.02 %) at 240 nm
Limit of quantification:	4 ng (0.08 %) at 240 nm

The same result, < 0.08 % impurities, was observed for ICRS 192171.

Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectrum of the main peak, given in Figure 3, showed a UV-maximum at 240 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

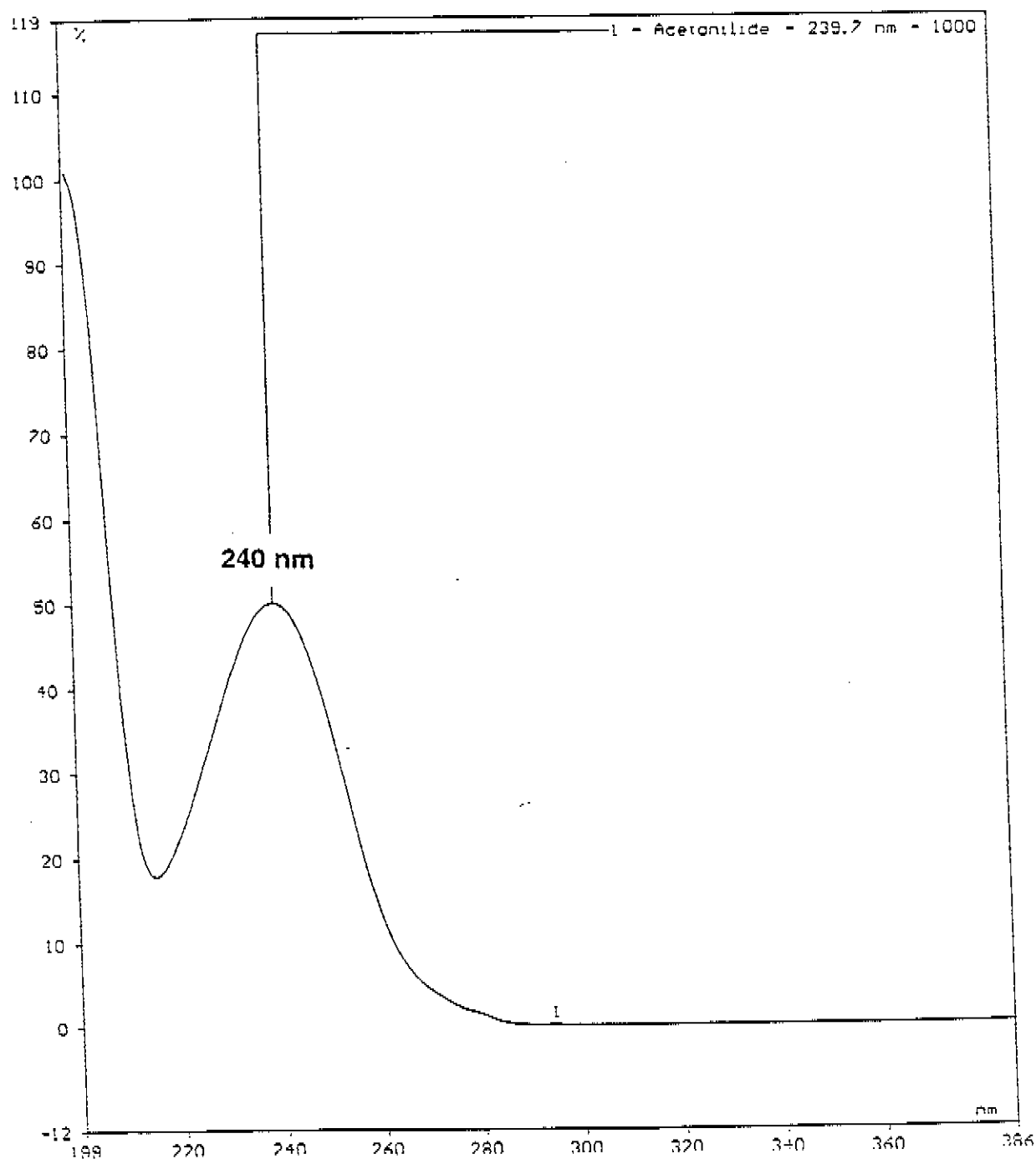


Figure 3. *UV-spectrum of acetanilide recorded in the eluent.*

Capillary electrophoresis

No impurities above the limit of quantification (0.15 %) were found.

An electropherogram is given in Figure 4.

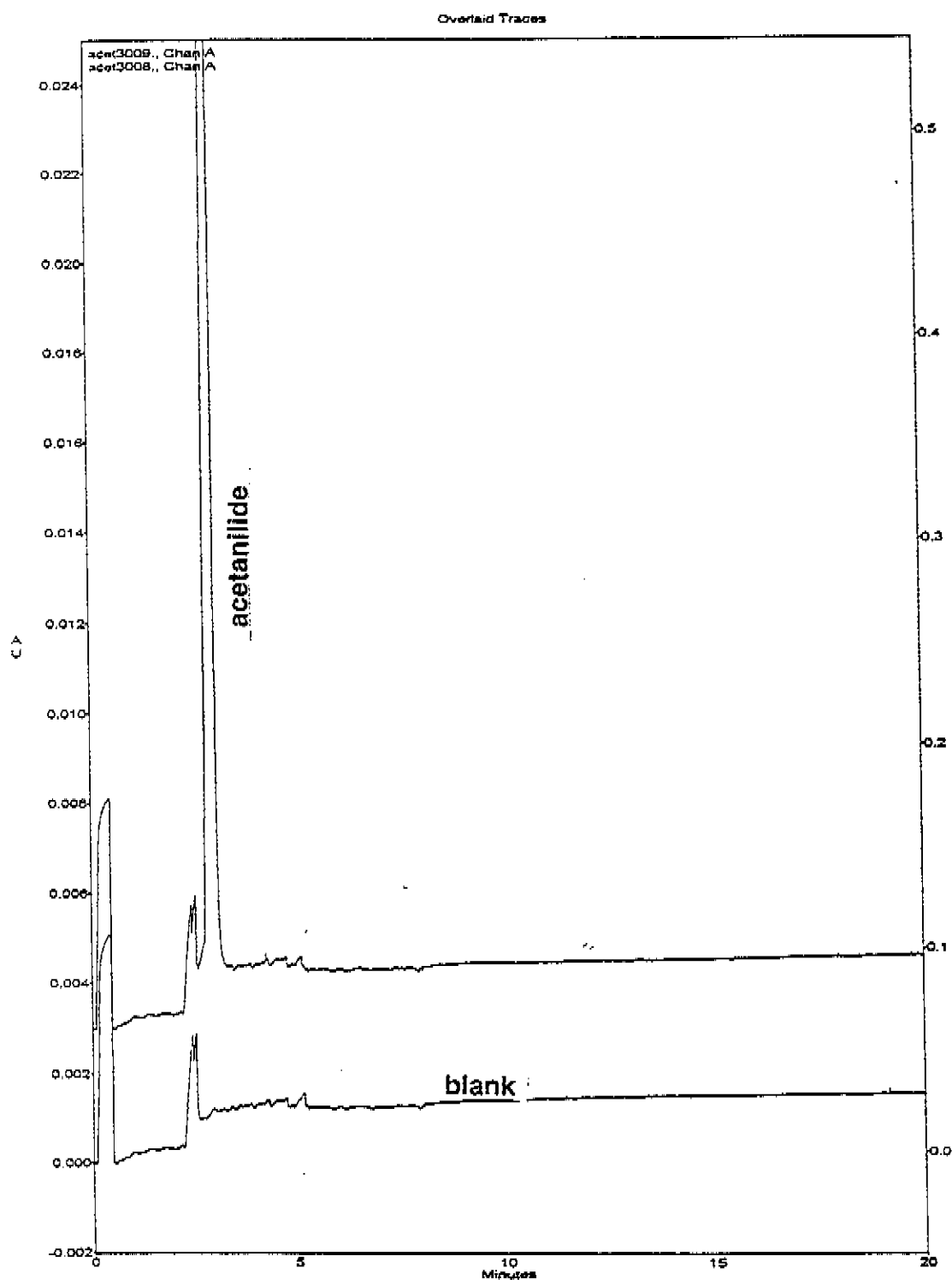


Figure 4. Electropherogram of acetanilide Control No 297171 monitored at 240 nm.

Eluent:	11.7 mM borate, 8.3 mM phosphate and 50 mM SDS at pH 9.20
Capillary:	Fused silica (total length 67 cm, length to detector 60 cm, ID: 75 μ m)
Detector:	Beckman P/ACE Diode Array Detector operated at 240 nm and 200 nm.
CE Instrument:	Beckman P/ACE system 5510 operated with an applied voltage of 30 kV for 20 minutes.
Run temperature:	50 °C.
Injection:	10 seconds pressure injection.
Integrator:	Beckman P/ACE Station Software.
Sample:	Acetanilide was dissolved in water at a concentration of 1.0 mg/ml. 10 seconds high pressure injection corresponds to 59 ng injected sample.
Stability in the eluent:	A sample was stored for 2 days. No signs of degradation were observed.
Limit of detection:	0.05 % at 240 nm
Limit of quantification:	0.15 % at 240 nm

Differential scanning calorimetry

The purity was estimated to about 99.97 mol % (n=10, RSD=0.02 %) determined by differential scanning calorimetry. The determination was performed on 2 mg using a heating rate of 2 °C per minute.

Melting temperature (T_M):	113.9 °C (n=10, RSD=0.1 %)
Onset:	113.7 °C (n=10, RSD=0.09 %)
Instrument:	Perkin Elmer DSC7 Differential Scanning Calorimeter

No signs of polymorphic forms were found when the substance was heated from 40 °C to 200 °C.

The previous batch ICRS 192171 showed a purity of 99.95 mol %.

Data given by the manufacturer

Identification IR:	Conforms.
Assay:	98.5-101.0 %
Anilin:	< 0.01 %
Phenol:	< 0.002 %
Sulphated ash:	< 0.1 %
Water:	< 0.2 %

Collaborative study

The melting point was assigned after a collaborative study including four laboratories. The melting point was determined according to *The International Pharmacopoeia*, Third Edition, Volume 1, as a capillary melting point determination.

Participating laboratories:

- Lab 1: Björn Egil Olsen
Norsk Medicinaldepot AS
Sven Oftedalsvej 10
PO Box 100
N 0518 OSLO
NORWAY
- Lab 2: Camilla Alvesson
Mettler Toledo AB
Hängpilsgatan 56
42677 Västra Frölunda
GÖTEBORG
SWEDEN
- Lab 3: Dr Ulrich Rose
Service de la Qualité du Médicament
European Pharmacopoeia Laboratory
B.P. 907
F-67029 Strasbourg Cedex 1
FRANCE
- Lab 4: WHO Collaborating Centre for Chemical Reference Substances
STOCKHOLM
SWEDEN

RESULTS

Method	Lab 1	Lab 2	Lab 3	Lab 4	Mean
Capillary melting point, °C	115.7	115.5	116.0	115.5	115.7
n	6	6	6	6	4
RSD, %	0.07	0.1	0.3	0.3	0.3

Assigned melting point: 116 °C

Stability

No special stability studies were performed as it was considered that this substance, based on the experience of the stability of the previous lot, was stable and showed no signs of degradation when stored for 34 years at + 20 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

Conclusion

Acetanilide, Control No 297171, can be considered suitable as WHO Melting Point Reference Substance for the intended purpose with the melting point set to 116 °C.

Captopril

Control No 197214

Analytical Report

Intended use

The monograph for Captopril in *The International Pharmacopoeia*, Third Edition, Volume 5, requires a reference substance of captopril to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity as well as in the liquid chromatographic system suitability test under related substances.

Material

About 100 g of the sample (manufacturers batch no SQ 14225 Capto-1-Re) were received at the WHO Centre in November 1997. The material is being stored in tightly closed containers at + 5 °C, protected from light.

Analytical data

Description

A white powder, with characteristic sulfide-odour.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W970028T). The spectrum is concordant with the spectrum of the United States Pharmacopeia Reference Standard (USPRS) lot H of captopril.

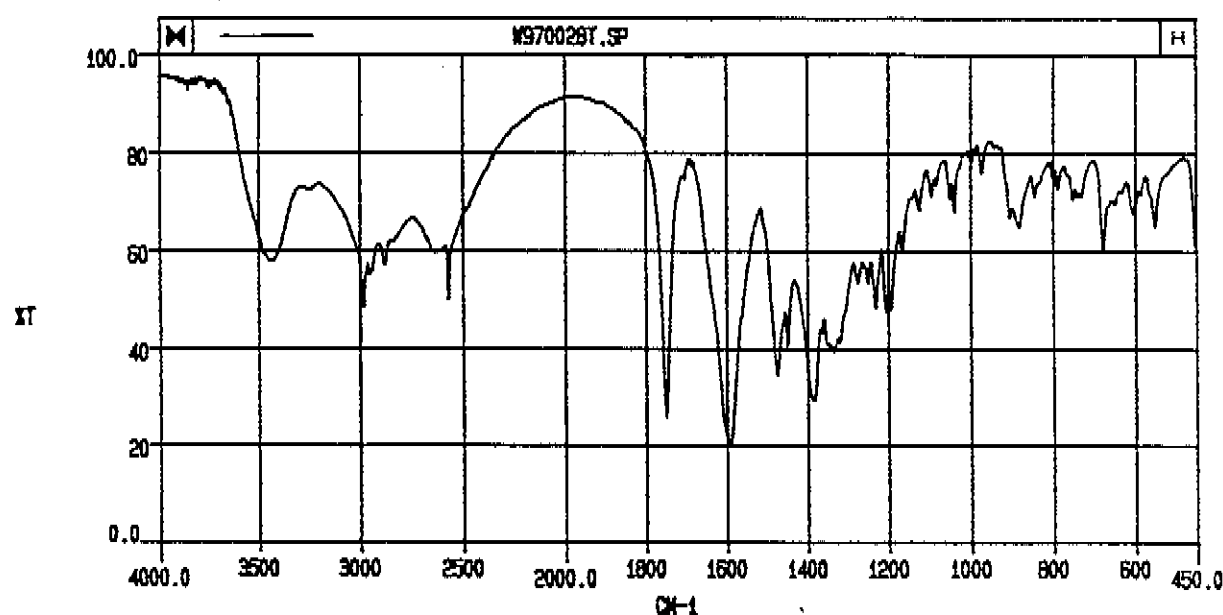


Figure 1. IR-spectrum of 1.1 mg of captopril Control No 197214 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of the main peak (captopril) was recorded by atmospheric pressure chemical ionization (APCI) in the positive ion mode. The spectrum, given in Figure 2, shows an $[M+H]^+$ ion of 218.2, which supports the identity of captopril.

Eluent:	Methanol:0.5 % glacial acetic acid (44:56)
Column:	Kromasil, C18, 4.6x150 mm, 5 μ m particles
Column temperature:	40 °C
Pump:	Hewlett Packard 1050 operated at a flow rate of 1 ml/min. After splitting the flow rate into the mass spectrometer was 0.1 ml/min.
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Cone voltage 20 V Source temperature 180 °C
Sample:	Captopril was dissolved in the eluent at a concentration of 1 mg/ml. After splitting about 2 μ g reached the mass spectrometer.

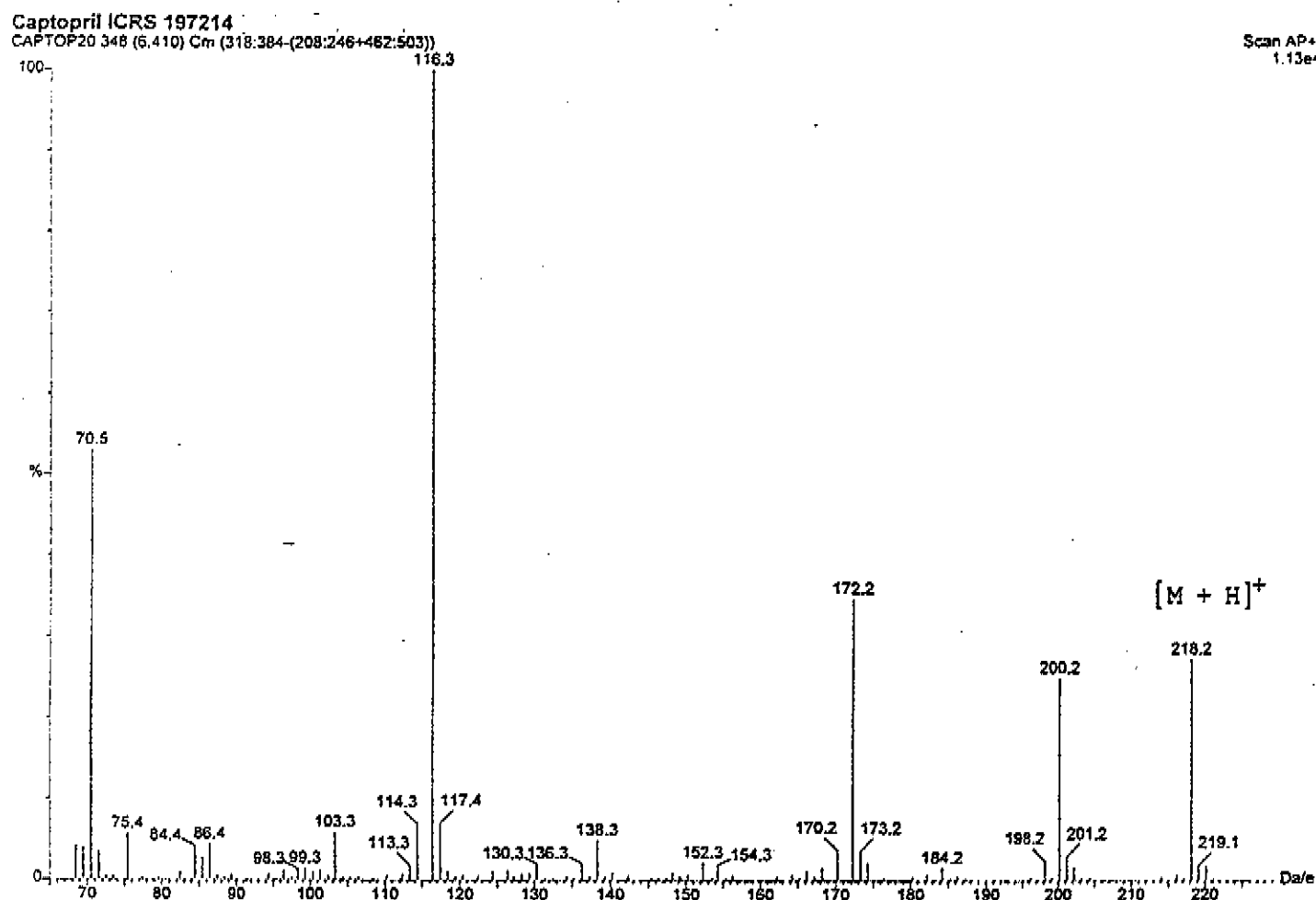


Figure 2. *Positive-ion atmospheric pressure chemical ionization mass spectrum of captopril Control No 197214.*

UV-spectrum

A UV-spectrum in 0.1 M NaOH was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 3.

A shoulder was observed around 235 nm.

$A_{1\text{cm}}^{1\%} = 259$ at 235 nm ($n=6$, $RSD=0.6\%$), calculated with reference to the dried substance.

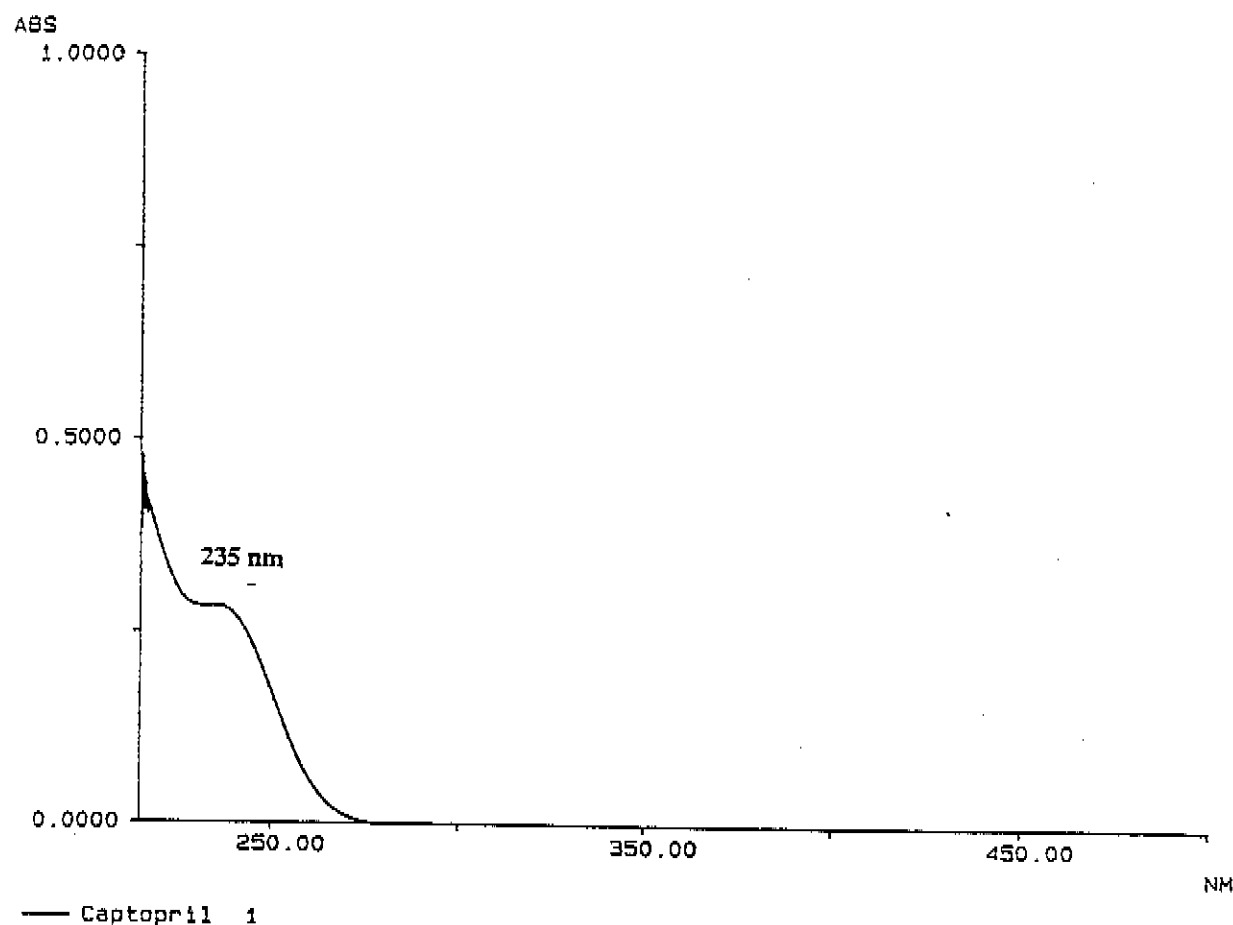


Figure 3. UV-spectrum of captopril Control No 197214, 11 µg/ml in 0.1 M NaOH.

Thin-layer chromatography

For the identity of captopril see results under Purity/Thin-layer chromatography.

Assay

Spectrophotometric assay

99.8 % (n=6, RSD=0.6 %). The determination was performed according to the method described above under Evidence of chemical structure/UV-spectrum. The USPRS lot H of captopril was used as standard and regarded as 100 %. The difference between the proposed ICRS and the USPRS lot H is, statistically, not significant at the 95 % confidence level using unpaired t-test.

Thermogravimetric analysis

This technique was not suitable to use as stabilization of weight during the analysis never occurred. Captopril melts at 107 °C. Loss on drying was used instead.

Water

It was not possible to use Karl Fischer titration as captopril reacted with the reagent resulting in values that are too high.

Loss on drying

0.1 % determined at 60 °C under reduced pressure over phosphorus pentoxide.

Organic volatile compounds

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. They were all estimated to be < 100 ppm.

The content of organic solvents was tested by gas chromatography with the following conditions:

Instrument:	Hewlett Packard 6890
Column:	HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas:	Helium (8 ml/min)
Detector:	FID
Injection volume:	2 µl
Injector temperature:	200 °C
Detector temperature:	250 °C
Temperature program:	40 °C for 10 minutes, 40 °C/min and holding 240 °C for 5 minutes.
Sample:	20 mg were shaken with 1 ml of benzyl alcohol and another 20 mg were shaken with 1 ml of Milli Q purified water each for about five minutes. The sample shaken with benzyl alcohol was filtered.

Purity

3-Mercapto-2-methylpropanoic acid: < 0.01 % determined by gas chromatography according to *The International Pharmacopoeia*, Third Edition, Volume 5.

Thin-layer chromatography

One secondary spot, with the same R_f as captopril disulfide, was detected. When determined against an external standard its amount was roughly estimated to 0.7 %. The following thin-layer chromatographic system according to *The International Pharmacopoeia*, Third Edition, Volume 5, was used.

Thin-layer:	Silica gel 60 F-254 (Merck) TLC and HPTLC
Eluent:	Toluene:glacial acetic acid:methanol (75:25:1)
Sample:	100 µg of captopril dissolved in methanol were applied.
Visualization:	Scanning at 200 nm with Camag TLC-scanner 3 was performed as well as visualization in day-light after spraying with 2, 2'-dinitro-5,5'-dithiodibenzoic acid/methanol.

R_f (captopril)=0.3 (TLC)/0.4 (HPTLC)

R_f (captopril disulfide)=0.1 (TLC/HPTLC)

The limit of detection of the impurity captopril disulfide was about 0.1 μg (0.1 %) when scanning at 200 nm.

The spot of captopril corresponds in position and appearance with that of the USPRS lot H of captopril.

High performance liquid chromatography

One impurity above the limit of quantification was found. It was identified by LC-MS as captopril disulfide and estimated to be 0.5 % ($n=6$, $\text{RSD}=10.8\%$) against an external standard. A chromatogram is shown in Figure 4.

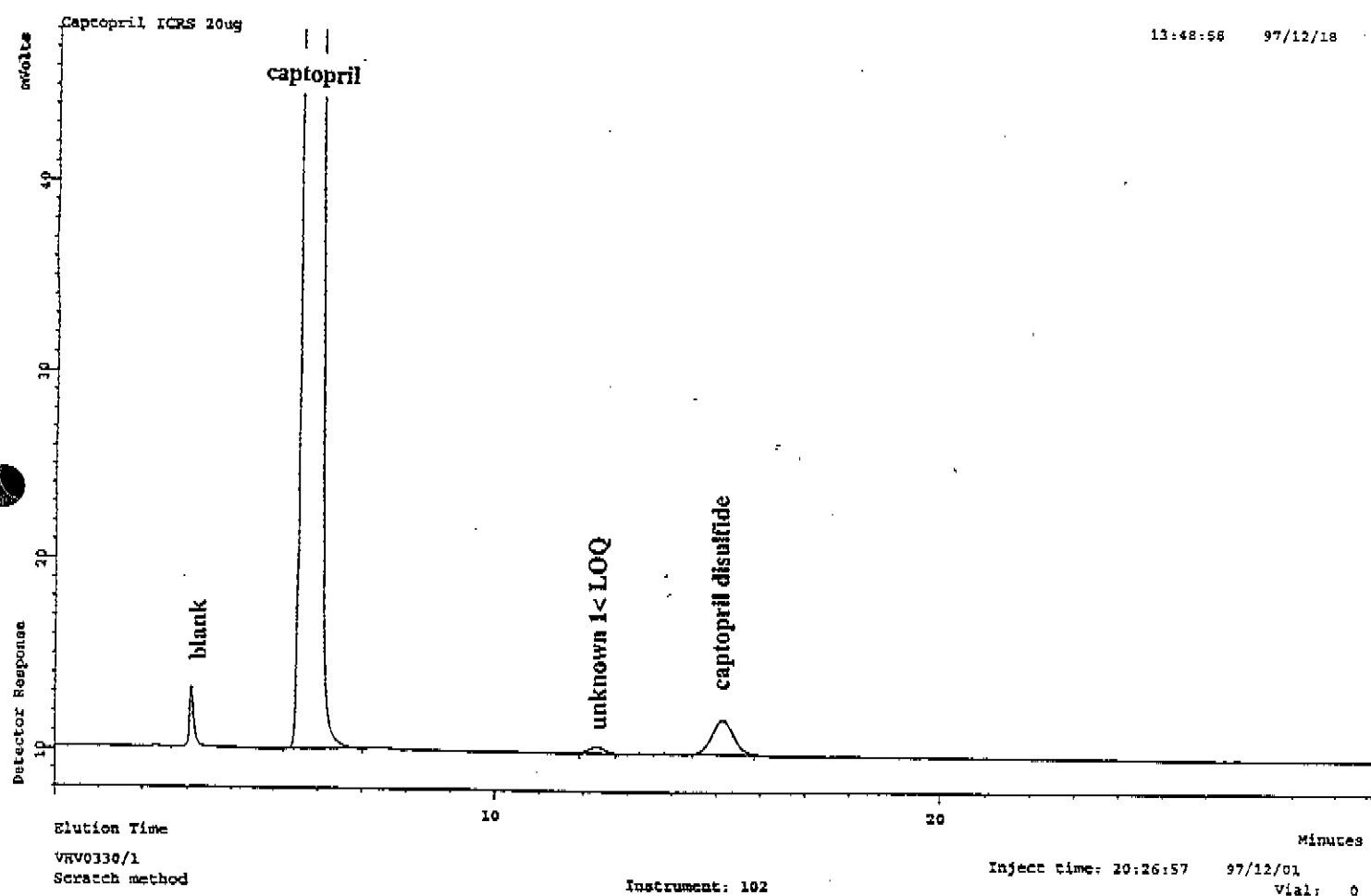


Figure 4. Chromatogram of captopril Control No 197214 monitored at 200 nm.

The following conditions were used:

Eluent:	Methanol:0.1 % concentrated phosphoric acid in water pH 2.1 (44:56, mixed by hand).
Column:	Kromasil, C8, 4.6 x 250 mm, 5 µm particles
Column temperature:	40 °C
Detector:	Varian Polychrom 9065 operated at 200 nm.
Pump:	Varian 9012 operated at a flow rate of 1.0 ml/min.
Injector:	Carnegie Med. CMA/200 operated at 8 °C.
Integrator:	PeakPro (Beckman)
Sample:	Captopril was dissolved in the eluent at a concentration of 1.0 mg/ml. 20 µl corresponding to 20 µg were injected.
Stability in the eluent:	When a sample was stored for 24 hours in the dark at 8 °C, a degradation of 1.3 % was observed. The solutions should be analyzed within two hours, when stored at 8 °C.
Limit of detection:	5 ng (0.03 %) at 200 nm
Limit of quantification:	17 ng (0.09 %) at 200 nm

Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of the impurity peaks, as well as the main peak, were similar with UV-maxima at about 200 nm. The wavelength of 200 nm was chosen since in the purity method, it gave the best sensitivity for all peaks. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities. One possible impurity, 2-methyl-3-acetylthiopropionic acid, has another UV-spectrum with a maximum around 230 nm. This impurity was, however, not found in this ICRS.

Impurities identified by LC-MS

The method described under Evidence of chemical structure/High-performance liquid chromatography with mass-spectrometric detection was used to identify the impurity eluting at 15 minutes in Figure 4. The impurity was identified as captopril disulfide, which was also verified by an external standard.

Differential scanning calorimetry

The purity was estimated to about 99.5 mol % (n=6, RSD=0.04 %). The determination was performed on 2 mg using a heating rate of 2 °C per minute.

Melting temperature (T _M):	106.7 °C (n=6, RSD=0.05 %)
Onset:	105.6 °C (n=6, RSD=0.06 %)
Instrument:	Perkin Elmer DSC7 Differential Scanning Calorimeter

No polymorphism was found, when measuring between 40 and 125 °C.

Ion chromatography

The ICRS was screened for the following anions: chlorides, bromides, nitrates, phosphates and sulfates. < 10 ppm of bromides, < 30 ppm of nitrates and < 100 ppm of sulfates were found. Chlorides and phosphates were not possible to determine as they co-elute with captopril.

The following conditions were used:

Eluent 1:	Sodium carbonate/sodium bicarbonate 1.8 mM/ 1.7 mM
Eluent 2:	Sodium bicarbonate (for the determination of chloride) 2.5 mM
Column:	Dionex HPIC AS4A
Suppressor:	ASRS-1
Detector:	Dionex 2000i, conductivity
Range:	3 μ S
Pump:	Dionex 2000i
Injector:	Beckman System Gold Autoinjector 507
Integrator:	PeakPro (Beckman)
Sample:	Captopril was dissolved in the eluent at a concentration of 1.0 mg/ml in water. 50 μ l corresponding to 50 μ g were injected.

Ion	Chlorides	Bromides	Nitrates	Phosphates	Sulfates
Limit of detection, ppm in the sample:	10	10	30	100	30
Limit of quantification, ppm in the sample:	50	50	100	500	100

Data given by the manufacturer

Identification IR:	Spectrum is consistent with structure.
Assay HPLC:	99.5 % "as is"
Iodometric titration:	99.5 % "dried basis", 99.4 % "as is"
Chromatographic purity (HPLC):	
Captopril disulfide	0.4 %
Individual impurities	Complies
Unknown	Complies
Total other impurities	Complies

Chromatographic impurities

(TLC):

Captopril disulfide < 0.5 %

L-Proline (SQ24836) ND

SQ 14224 ND

SQ 25487 (2-methyl-3-acetylthiopropionic acid) ND

3-Mercapto-2-Methylpropanoic Acid

(GC): 0.001 %

Loss on drying: 0.06 %

Melting point: 108 °C

Specific optical rotation: -129°

Heavy metals: < 30 ppm

Residue on ignition: 0.0 %

Appearance: Crystalline powder, practically free from visible impurities

Color: White to off-white

Odor: Characteristic sulfide-odor

Stability

Regular re-examinations of the ICRS when stored in the dry state will be performed.

Conclusion

Captopril, Control No 197214, can be considered suitable as International Chemical Reference Substance for the intended purpose.

Ciprofloxacin hydrochloride

Control No 197210

Analytical Report

Intended use

The monograph for Ciprofloxacin hydrochloride in *The International Pharmacopoeia*, Third Edition, Volume 5, requires a reference substance of ciprofloxacin hydrochloride to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity as well as in the liquid chromatographic assay. The monograph for Ciprofloxacin in *The International Pharmacopoeia*, Third Edition, Volume 5, requires a reference substance of ciprofloxacin hydrochloride to be used in the liquid chromatographic test for related substances.

Material

About 66 g of the sample (manufacturers batch no R-124-2) were received at the WHO Centre in July 1996. The material is being stored in tightly closed containers at + 5 °C, protected from light.

Analytical data

Description

A pale yellow powder.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W.197210T). The spectrum is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1 of ciprofloxacin hydrochloride.

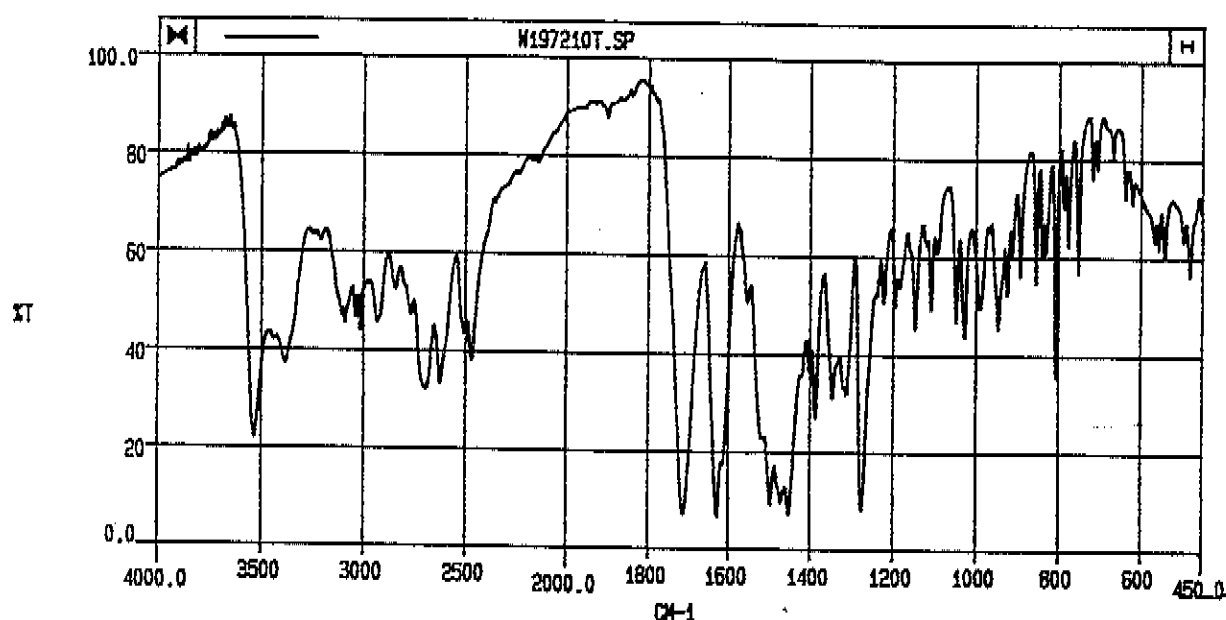


Figure 1. IR-spectrum of 1.0 mg of ciprofloxacin hydrochloride Control No 197210 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of ciprofloxacin was recorded by electrospray (ESI) in the positive ion mode. The spectrum shows an $[M + H]^+$ ion of 332.0, which supports the identity of ciprofloxacin. The spectrum, given in Figure 2, is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1 of ciprofloxacin hydrochloride.

Eluent:	Acetonitrile:1 % glacial acetic acid in water (50:50)
Column:	Direct inlet
Pump:	Hewlett Packard 1050 operated at a flow rate of 0.2 ml/min
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Cone voltage 65 V
	Source temperature 140 °C
Sample:	Ciprofloxacin hydrochloride was dissolved in the eluent at a concentration of 0.01 mg/ml. 20 µl corresponding to 200 ng were injected.

Ciprofloxacin HCl ICRS 197210 ESI+ 140 C CV65 ACN:1 % HAc (50:50)
CIPROFL 46 (3.935) Crt (40:54-(25:31+13:20))

100-

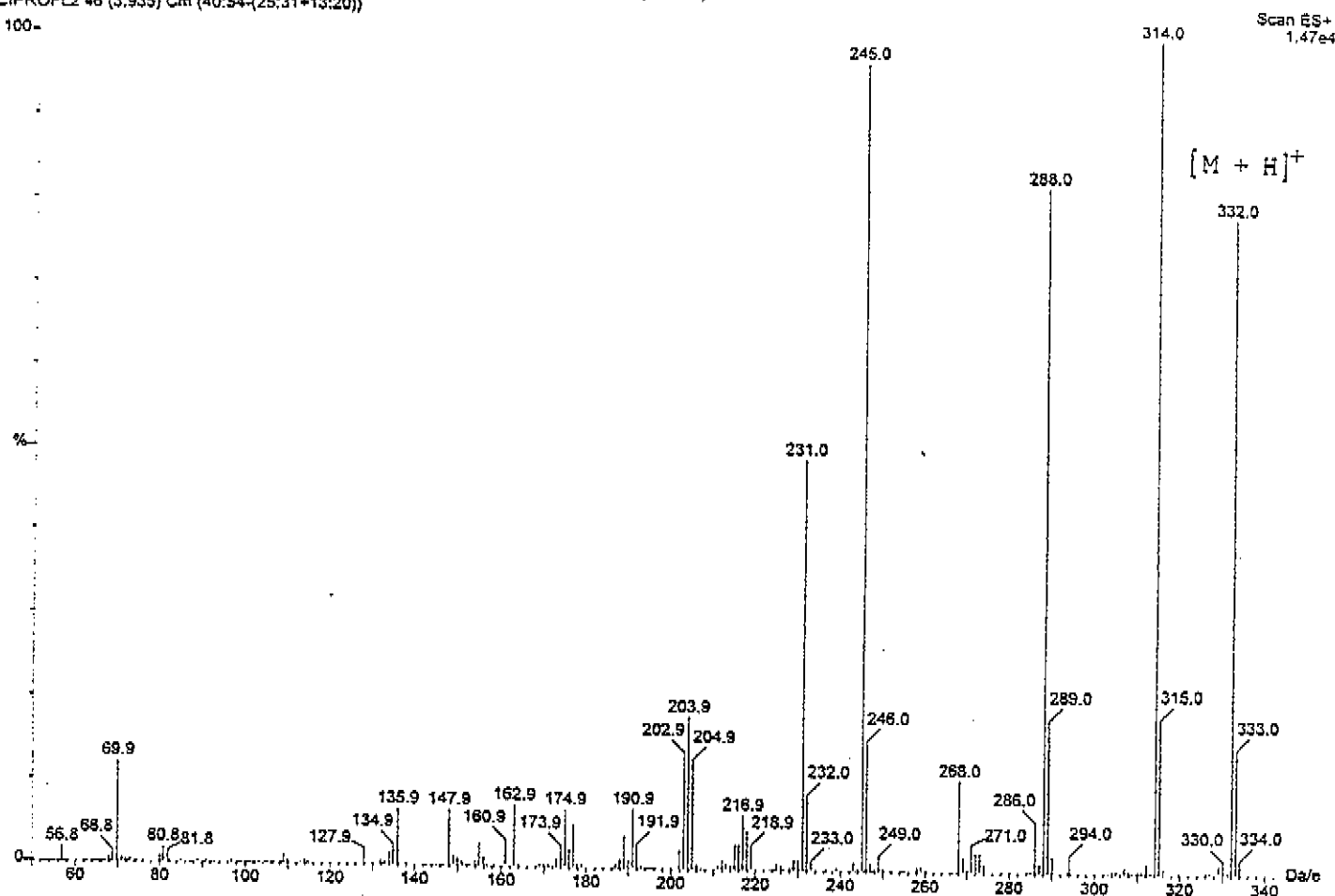


Figure 2. Positive-ion electrospray mass spectrum of ciprofloxacin hydrochloride Control No 197210.

UV-spectrum

A UV-spectrum in water was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 3.

A UV-maximum was observed at 275 nm.

A_{1cm}^{1%} = 1079 at 275 nm (n=6, RSD=0.4 %).

Calculations were performed with reference to the dried substance.

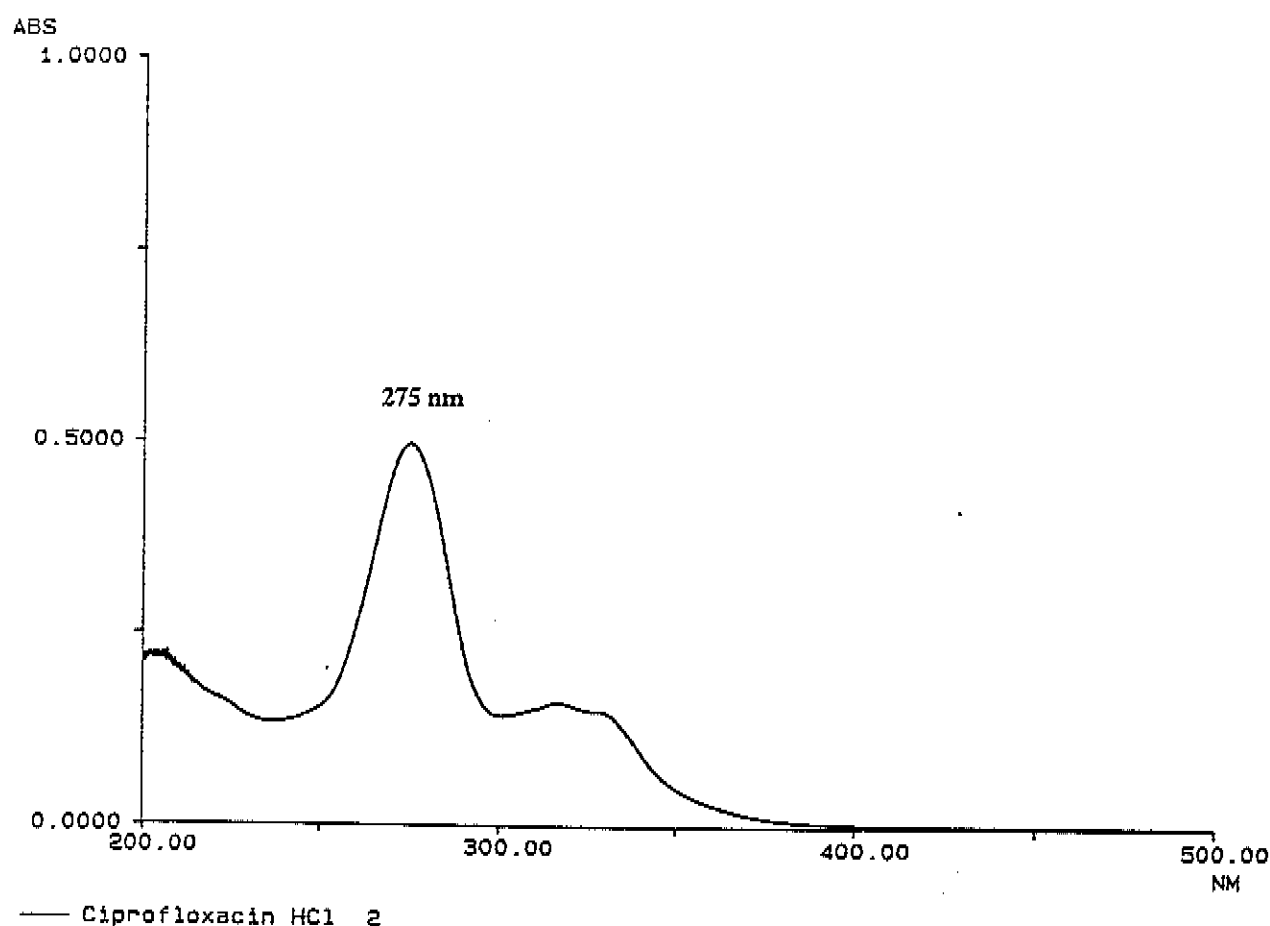


Figure 3. UV-spectrum of ciprofloxacin hydrochloride Control No 197210. 4.6 µg/ml in water.

Thin-layer chromatography

For the identity of ciprofloxacin hydrochloride see results under Purity/Thin-layer chromatography.

Chloride

For the identity and estimation of chloride see under Purity/Ion Chromatography.

Assay

Liquid chromatographic assay

100.5 % (n=6, RSD=0.5 %). The determination was performed at 278 nm according to the method described below under Purity/High performance liquid chromatography. The EPCRS lot 1 of ciprofloxacin hydrochloride was used as standard and regarded as 100 %. The result was calculated on the dried substances. The difference between the proposed ICRS and the EPCRS lot 1 is, statistically, not significant at the 95 % confidence level using unpaired t-test.

Spectrophotometric assay

100.0 % (n=6, RSD=0.4 %). The determination was performed according to the method described above under Evidence of chemical structure/UV-spectrum. The EPCRS lot 1 of ciprofloxacin hydrochloride was used as standard and regarded as 100 %. Calculations were performed with reference to the dried substances.

Thermogravimetric analysis

When the substance was heated to 120 °C, a loss of 6.0 % (w/w) was observed (n=6, RSD=2.2 %).

Instrument: Perkin-Elmer TGA 7
Sample weight: 3 mg
Heating program: 5 °C/min from 20-120 °C and then holding 120 °C for 40 minutes
Melting point: 255-257 °C (decomposition) for ciprofloxacin

The corresponding result for the EPCRS lot 1 was 6.4 % (w/w), (n=6, RSD=0.9 %).

Water

6.1 % (n=2) determined by Karl Fischer titration.

Organic volatile compounds

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them were estimated to be <100 ppm.

The content of organic solvents was tested by gas chromatography with the following conditions:

Instrument: Hewlett Packard 6890
Column: HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas: Helium (8 ml/min)
Detector: FID
Injection volume: 2 µl
Injector temperature: 200 °C
Detector temperature: 250 °C
Temperature program: 40 °C for 10 minutes, 40 °C/min and holding 240 °C for 5 minutes.
Sample: 20 mg were shaken with 1 ml of benzyl alcohol and another 20 mg were shaken with 1 ml of Milli Q purified water each for about five minutes. If undissolved particles were found, filtration was performed.

PurityThin-layer chromatography

One secondary spot, probably the ethylenediamine-compound was detected, but estimated to be < limit of quantification (0.1 %). No detectable amounts of fluoroquinolonic acid were found, not even when scanning at 254 nm where a higher UV-absorbance was obtained. The following thin-layer chromatographic system according to *The International Pharmacopoeia*, Third Edition, Volume 5 was used.

Thin-layer: Silica gel 60 F-254 (Merck) TLC.
Eluent: Acetonitrile:ammonia (260g/l):methanol:dichloromethane
(1:2:4:4)
Sample: 100 and 50 µg of ciprofloxacin hydrochloride dissolved in
acetic acid were applied.
Visualization: Scanning at 278 nm with a CAMAG TLC Scanner 3 was
performed as well as visualization in UV-light of 254 nm.

R_f (ciprofloxacin hydrochloride)=0.54

R_f (fluoroquinolonic acid)=0.68

R_f (desfluoro-compound)=0.47

R_f (ethylenediamine-compound)=0.43

R_f (by-compound A)=0.49

The spot of ciprofloxacin hydrochloride corresponds in position and appearance with that of the EPCRS lot 1 of ciprofloxacin hydrochloride.

No secondary spots were detected in EPCRS lot 1.

High performance liquid chromatography

No impurities above the limit of quantification were found, which means < 0.1 % when determined by peak area normalization. External reference substances of four possible impurities were injected. They were all estimated to be below the limit of detection or quantification which means for fluoroquinolonic acid < 0.04 % (limit of detection), for the desfluoro-compound < 0.02 % (limit of detection), for the ethylenediamine-compound < 0.06 % (limit of quantification) and for by-compound A < 0.06 % (limit of detection).

A chromatogram is shown in Figure 4.

15:31:15 97/04/02

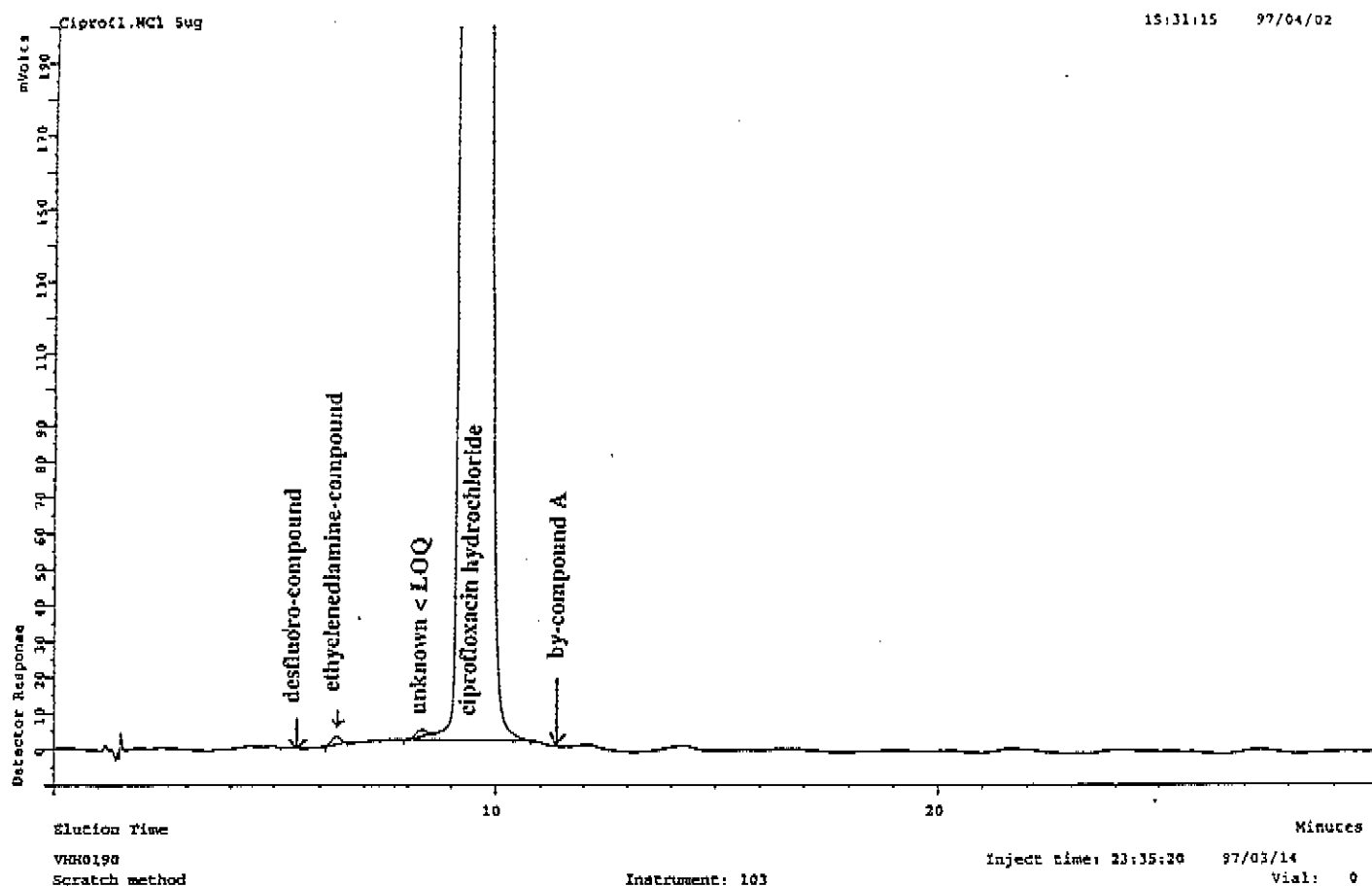


Figure 4. *Chromatogram of ciprofloxacin hydrochloride Control No 197210 monitored at 278 nm.*

The following conditions were used:

Eluent:	Acetonitrile:Phosphate buffer with triethylamine (10:90) Buffer: Phosphoric acid (2.45 g/l) mixed with triethylamine to get a pH of 3.0.
Column:	Genesis C18, 4 μ m, 4.6 x 150 mm
Column temperature:	40 °C
Detector:	Gynkotek UVD 340S operated at 278 nm.
Pump:	Gynkotek M480 operated at a flow rate of 1.5 ml/min.
Injector:	Gynkotek Gina 50 operated at 8 °C.
Integrator:	PeakPro (Beckman)
Sample:	Ciprofloxacin hydrochloride was dissolved in the eluent at a concentration of 0.5 mg/ml. 10 μ l corresponding to 5 μ g were injected.
Stability in the eluent:	A sample was stored for about 15 hours. No signs of degradation were observed.
Limit of detection:	2 ng (0.04 %) at 278 nm.
Limit of quantification:	5 ng (0.1 %) at 278 nm.

In this system fluoroquinolonic acid elutes late. In order to elute it within 30 minutes a gradient was used. The amount of fluoroquinolonic acid was determined by another liquid chromatographic method optimized for fluoroquinolonic acid.

The same result, < 0.1 % impurities, was observed for EPCRS lot 1.

Diode-array detection

The chromatographic system described above was used to record UV-spectra for the main peak of ciprofloxacin and for the potential impurities. Ciprofloxacin showed UV-maxima at 205 and 278 nm. Fluoroquinolonic acid showed main maxima at 255 and 262 nm, by-compound A at 255 and 270 nm, desfluoro-compound at 283 nm and the ethylenediamine-compound at 276 nm. The wavelength of 278 nm was chosen in the purity method. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities. The impurities mentioned will be available as separate impurity reference substances.

Differential scanning calorimetry

The thermogram of ciprofloxacin hydrochloride showed a small exotherm at about 160 °C which indicates the presence of another polymorphic form. The melting temperature (peak maximum) is about 325 °C for ciprofloxacin hydrochloride.

Similar results were obtained for the EPCRS lot 1.

Ion chromatography

The ICRS was screened for the following anions: chlorides, bromides, nitrates, phosphates and sulfates. Less than 10 ppm of bromides, 100 ppm of nitrates and less than 500 ppm of phosphates were found. Sulfates were found and estimated to 350 ppm. Chlorides were found and roughly estimated to be 9.7 % (theoretical value 9.2 %).

The following conditions were used:

Eluent 1:	Sodium carbonate/sodium bicarbonate 1.8 mM/ 1.7 mM
Eluent 2:	Sodium bicarbonate (for the determination of chloride) 2.5 mM
Column:	Dionex HPIC AS4A
Suppressor:	ASRS-1
Detector:	Dionex 2000i, conductivity
Range:	3 µS (for determination of chloride 300 µS)
Pump:	Dionex 2000i
Injector:	Beckman System Gold Autoinjector 507
Integrator:	PeakPro (Beckman)
Sample:	Ciprofloxacin hydrochloride was dissolved in the eluent at a concentration of 1.0 mg/ml in water. 50 µl corresponding to 50 µg were injected.

Ion	Chlorides	Bromides	Nitrates	Phosphates	Sulfates
Limit of detection, ppm in the sample:	10	10	30	100	30
Limit of quantification, ppm in the sample:	50	50	100	500	100

Data given by the manufacturer

Appearance:	Complies
Identity IR:	Complies
Identity TLC:	Complies
Identity chloride:	Complies
Clarity of solution:	Complies
Colour of solution:	Complies
pH value:	3.8 (limits 3.0-4.5)
Sulfate:	< 200 ppm (limit max 400 ppm)
Heavy metals:	< 10 ppm (limit max 20 ppm)
Water:	6.03 % (limits 4.7-6.7 %)
Sulfated ash:	< 0.05 % (limit max 0.1 %)
By-products HPLC	
Fluoroquinolonic acid:	< 0.05 % (limit max 0.2 %)
By-product A	< 0.1 % (limit max 0.2 %)
Desfluoro-compound	< 0.05 % (limit max 0.2 %)
Ethylenediamine-compound	< 0.05 % (limit max 0.2 %)
Other by-products individual:	< 0.05 % (limit max 0.2 %)
Sum of by-products:	0.1 % (limit max 0.5 %)
Assay, calculated on the dried substance:	99.9 % (limit 98.0-102.0 %)

Stability

Stability studies were not performed as this substance was not suspected to degrade easily according to WHO/PHARM/86.529. Regular re-examinations of the ICRS when stored in the dry state will be performed.

Conclusion

Ciprofloxacin hydrochloride, Control No 197210, can be considered suitable as International Chemical Reference Substance for the intended purpose. When used in the liquid chromatographic assay according to *The International Pharmacopoeia*, Third Edition, Volume 5, the content of ciprofloxacin hydrochloride is taken to be 100.0 % calculated with reference to the dried substance which corresponds to 94.0 % on the "as is" basis.

Cisplatin

Control No 197207

Analytical Report

Intended use

The monograph for Cisplatin in *The International Pharmacopoeia*, Third Edition, Volume 4, requires a reference substance of cisplatin to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity as well as in the spectrophotometric assay.

Material

About 10 g of the sample (manufacturers batch no 89 730 021/33/94) were received at the WHO Centre in June 1995. The material is being stored in tightly closed containers at + 5 °C, protected from light.

CAUTION: As cisplatin is a cytotoxic drug it should be handled with care. Avoid contact with the skin and inhalation of airborne particles.

Analytical data

Description

A yellow crystalline powder.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W197207). The spectrum is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1 of cisplatin.

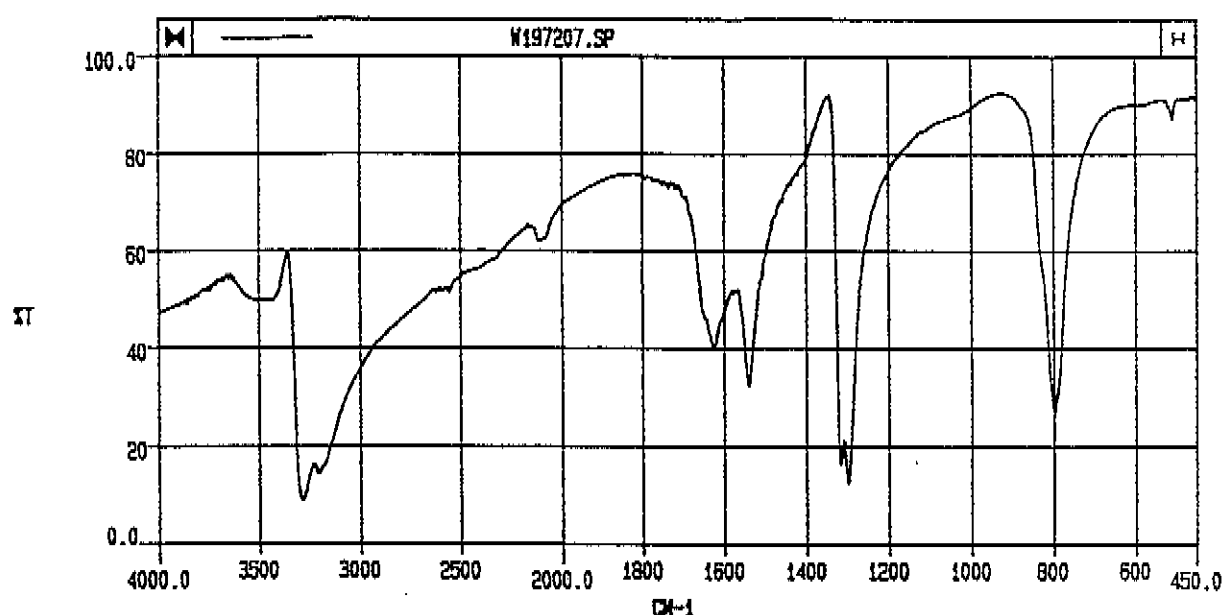


Figure 1. IR-spectrum of 1 mg of cisplatin Control No 197207 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

High-performance liquid chromatography with mass-spectrometric detection

A spectrum of the main peak (cisplatin) was recorded by electrospray (ESI) in the positive ion mode. The spectrum shows an $[M + H]^+$ ion of 300.9, which supports the identity of cisplatin. The spectrum is given in Figure 2. The EPCRS lot 1 of cisplatin shows a similar mass spectrum as the ICRS 197207.

Eluent:	Water 100 %
Column:	Hypercarb, 100 mm (Shandon)
Pump:	Hewlett Packard 1050 operated at a flow rate of 1 ml/min. After splitting, the flow rate into the mass spectrometer was about 0.07 ml/min.
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Cone voltage 35 V Source temperature 150 °C
Sample:	Cisplatin was dissolved in the eluent at a concentration of 1 mg/ml. After splitting about 1 µg was injected into the mass spectrometer.

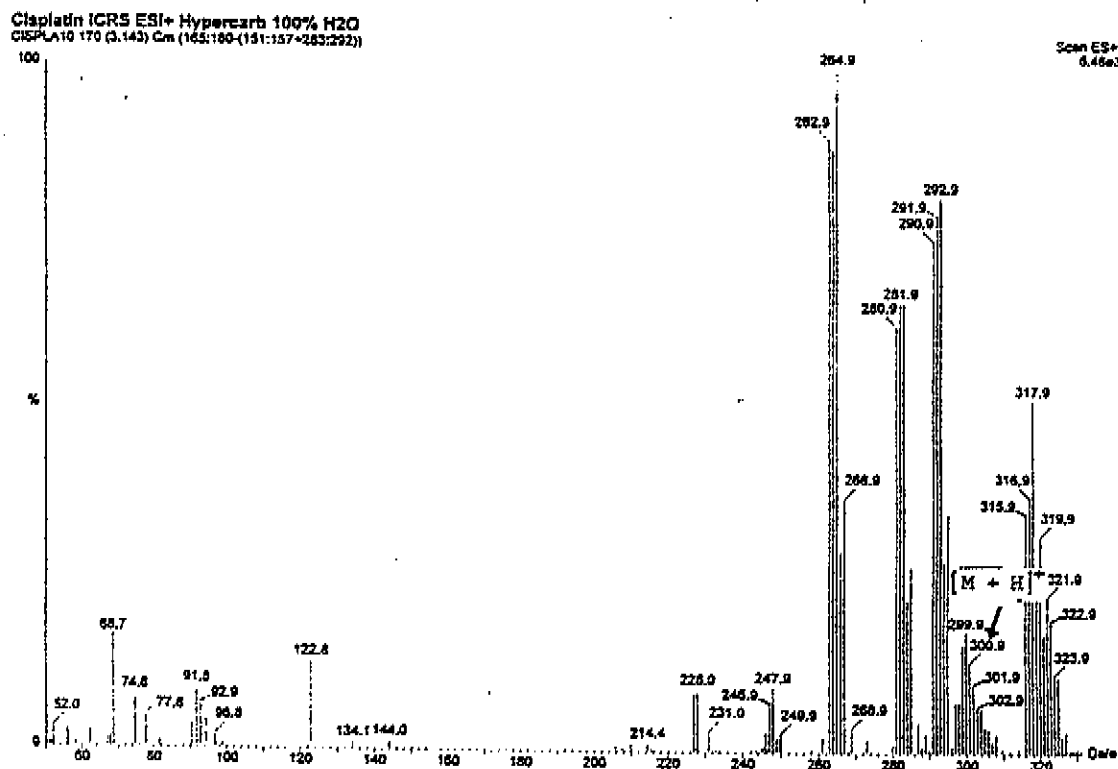


Figure 2. Positive-ion electrospray mass spectrum of cisplatin Control No 197207.

Identification of a degradation product in solution

The chromatographic system described above was used to check the identity of a peak appearing when cisplatin was stored in an aqueous solution. A peak eluting at about 2 minutes was identified as the monohydrated complex with an $[M + H]^+$ ion of 284.

Cisplatin elutes at about 2.9 minutes. One potential impurity, transplatin, was also injected and found to elute at about 5.6 minutes. It showed an $[M + H]^+$ ion of 300.9.

Mass spectrometry of the monohydrated complex has been reported by H. Ehrsson, I. Wallin, A. Andersson and P-O. Edlund; Cisplatin, transplatin and their hydrated complexes: Separation and identification using porous graphitic carbon and electrospray ionization mass spectrometry, *Anal. Chem.*, 67(1995)3608-3611.

Assay

See results from the manufacturer. Assay performed according to the European Pharmacopoeia and the United States Pharmacopoeia.

Thermogravimetric analysis

When the substance was heated to 100 °C, a loss of < 0.1 % (w/w) was observed (n=3).

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.

Sample weight: 2 mg.

Heating program: 5 °C/min from 20–100 °C and then holding 100 °C for 70 minutes.

Melting point: 270 °C with decomposition.

Water

See results from the manufacturer.

Purity

Thin-layer chromatography

See results from the manufacturer.

Capillary electrophoresis

Capillary electrophoresis was chosen for the purity determination as cisplatin and the two potential impurities transplatin and potassium trichloroaminoplatinate were possible to separate within reasonable time and to detect without derivatization. Only one impurity was found, namely the monohydrated complex which was estimated to 0.77 % by peak area normalization ($n=9$, $RSD=0.14$ % for the main peak, $RSD=10.4$ % calculated on the 0.77 % impurity level). It was earlier identified by LC-MS and found to increase rapidly in water solution. After 24 hours only about 60 % of intact cisplatin remains. For stability reasons it is better to prepare the sample in 0.5 % sodium chloride which is chosen in the method. In this solution a degradation of about 0.5 % was observed, when stored at 15 °C for 24 hours. Electropherograms are given in Figure 3 (full scale) and Figure 4 (details).

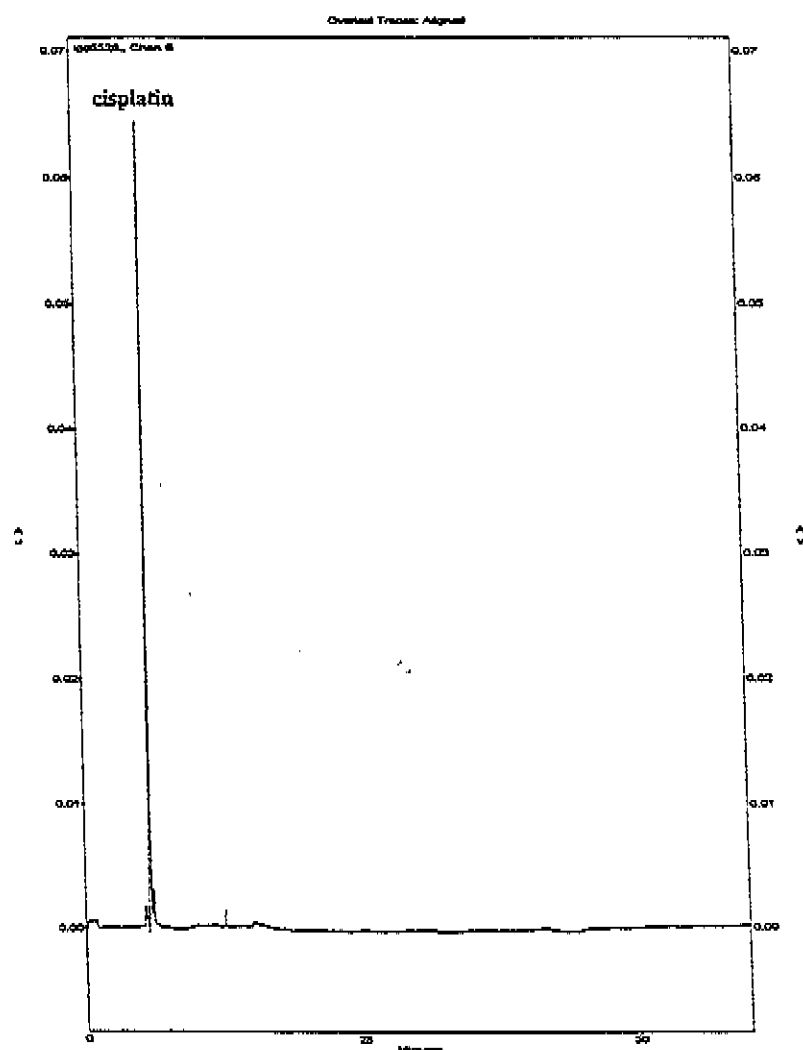


Figure 3. Electropherogram of cisplatin Control No 197207 monitored at 210 nm.

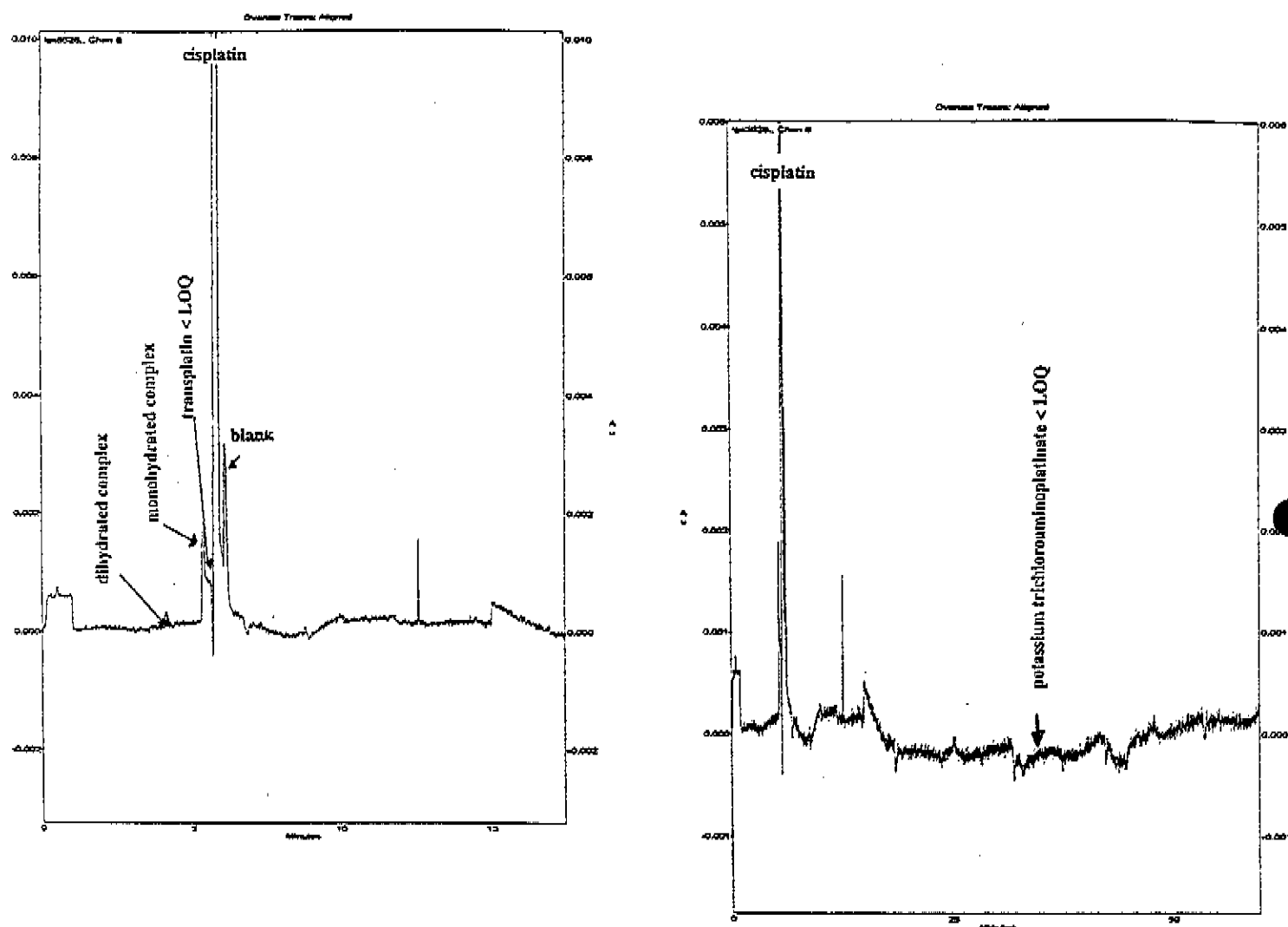


Figure 4. Electropherogram (details) of cisplatin Control No 197207 monitored at 210 nm.

Transplatin (limit of detection=1.3 %) and potassium trichloroaminoplatinate (limit of detection=0.8 %) were not found in this ICRS.

The following conditions were used:

Eluent:	100 mM boric acid + 50 mM phosphoric acid in water, pH adjusted to 7.0 with 2 M sodium hydroxide
Capillary:	Fused silica (total length 60 cm, length to detector 50 cm, ID: 50 µm)
Detector:	P/ACE System MDQ operated at 210 nm.
CE Instrument:	P/ACE System MDQ operated with an applied voltage of 20 kV for 60 minutes.
Run temperature:	25 °C
Sample temperature:	15 °C

Injection:	20 seconds pressure injection, at a pressure of 0.5 psi, which corresponds to about 24 nl.
Integrator:	PeakPro (Beckman)
Sample:	Cisplatin was dissolved in 0.5 % NaCl at a concentration of 1.0 mg/ml.
Stability in 0.5 % NaCl:	A sample was stored for 4 h at 15 °C. No signs of degradation were observed. After 24 h a degradation of about 0.5 % was observed.
Limit of detection:	0.4 % at 210 nm (Cisplatin) 1.3 % at 210 nm (Transplatin) 0.8 % at 210 nm (Potassium trichloroaminoplatinate)
Limit of quantification:	1.3 % at 210 nm (Cisplatin) 4.3 % at 210 nm (Transplatin) 2.5 % at 210 nm (Potassium trichloroaminoplatinate)

Silver content

See results from the manufacturer.

Platinum content

See results from the manufacturer.

Data given by the manufacturer

Pt content: 65.14 % (limit 64.9-65.2 %)

Characterization according to EP II.

Identification IR:	Meets requirements.
Identification TLC:	Meets requirements.
Appearance of solution S1:	Meets requirements.
pH of solution S1:	5.88 (limits 4.5-6.0)
Related substances:	Meets requirements.
Ag content:	18 ppm based on cisplatin (limit < 250 ppm)
Assay cisplatin:	100.12 % (limit 97.0-102.0 %)

Characterization according to USP XXIII.

Identification HPLC:	Meets requirements.
Identification IR:	Meets requirements.
Identification TLC:	Meets requirements.
Crystallinity:	Crystalline

Water content:	0.1 % (limit 1 %)
UV Purity, E 301/E 246:	5.12 (limit > 4.5)
Potassium trichloroaminoplatinate:	0.1 % (limit < 1%)
Transplatin:	0.06 % (limit < 2%)
Assay cisplatin:	100.77 % (limit >98 %)

Stability

Regular re-examinations of the ICRS when stored in the dry state will be performed.
For stability in solution please see under capillary electrophoresis.

Conclusion

Cisplatin, Control No 197207, can be considered suitable as International Chemical Reference Substance for the intended purpose. When used in the spectrophotometric assay according to *The International Pharmacopoeia*, Third Edition, Volume 4, the content of cisplatin is taken to be 100 % calculated with reference to the dried substance which corresponds to 100 % on the "as is" basis.

Kanamycin monosulfate (anhydrous)

Control No 197211

Analytical Report

Intended use

The monograph for Streptomycin sulfate powder for injections in *The International Pharmacopoeia*, Third Edition, Volume 4, requires a reference substance of kanamycin monosulfate to be used in the thin-layer chromatographic test for identity.

Material

About 3.6 g of the sample, dispensed in ampoules of about 12 mg/ampoule, were received from the National Institute for Biological Standards and Control in January 1998. The material is being stored at -20 °C, protected from light.

This ICRS is of the same origin as that of the 1st International Biological Standard for Kanamycin.

Analytical data

Description

A white and very fluffy powder.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum of the untreated substance is given in Figure 1 (No W960042T).

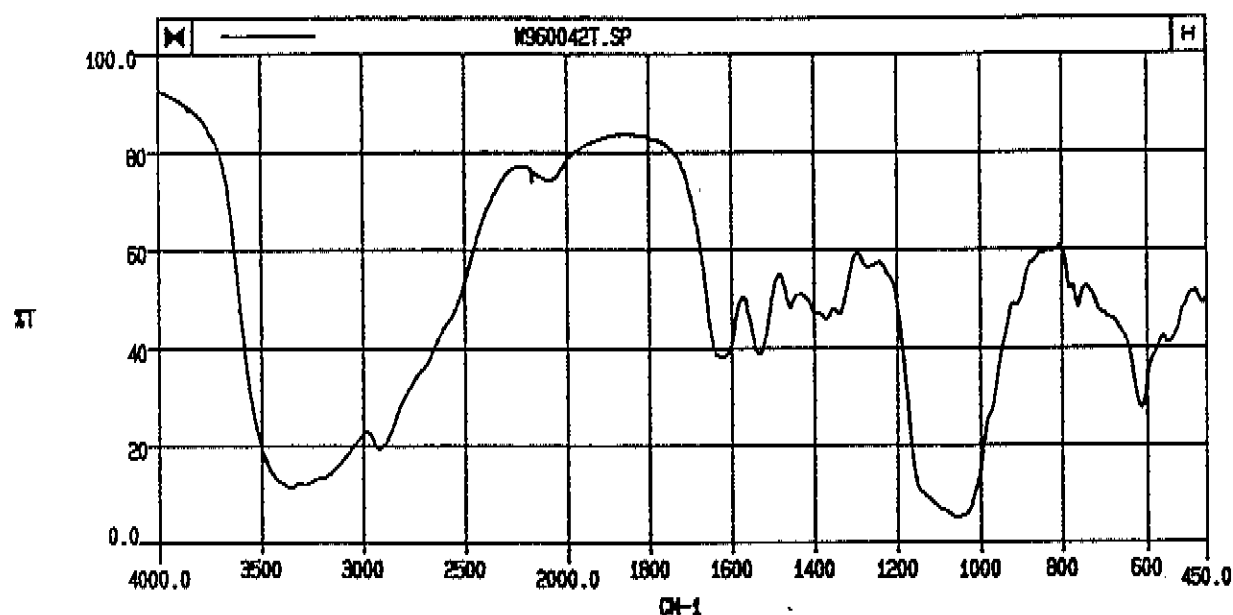


Figure 1. IR-spectrum of 1.5 mg of untreated kanamycin monosulfate Control No 197211 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

The spectrum in Figure 1 is concordant with the spectra of the United States Pharmacopeia Reference Standard (USPRS) lot I and of the European Pharmacopoeia Chemical Reference substance (EPCRS) batch 2 of kanamycin monosulfate when they are re-crystallized in water.

The proposed ICRS was also stored for 24 hours in 98 % relative humidity in order to pick up moisture to re-crystallize. Before preparing an IR-disc the substance was dried. An infrared spectrum of the substance after this re-crystallization is given in Figure 2 (960042RH).

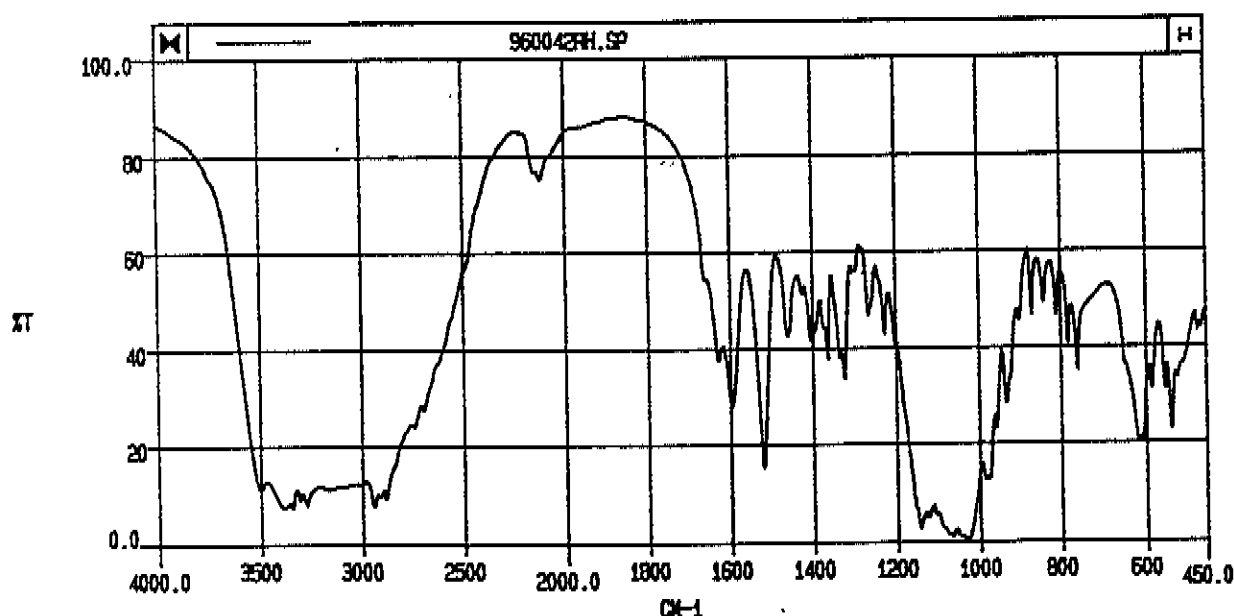


Figure 2. IR-spectrum of 1.8 mg moisture treated kanamycin monosulfate Control No 197211 in 300 mg of potassium bromide recorded against a potassium bromide disc.

The spectrum in Figure 2 is concordant with the spectra of the United States Pharmacopeia Reference Standard (USPRS) lot I and of the European Pharmacopoeia Chemical Reference substance (EPCRS) batch 2 of kanamycin monosulfate.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of kanamycin was recorded by electrospray (ESI) in the positive ion mode. The spectrum shows an $[M + H]^+$ ion of 485.1, which supports the identity of kanamycin. The spectrum, given in Figure 3, is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) batch 2.

Eluent:	Acetonitrile:1 % glacial acetic acid in water (50:50)
Column:	Direct inlet
Pump:	Hewlett Packard 1050 operated at a flow rate of 0.2 ml/min
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Cone voltage 25 V Source temperature 140 °C
Sample:	Kanamycin sulfate was dissolved in the eluent at a concentration of 0.01 mg/ml. 20 µl corresponding to 200 ng were injected.

Kanamycin sulfate ICRS 197211 ESI+ 140 C CV25 ACN:1 % HAc (50:50)
KANAMYCS 26 (2.235) Cm (21:35-(14:17+10))

Scan ES+
1.06e4

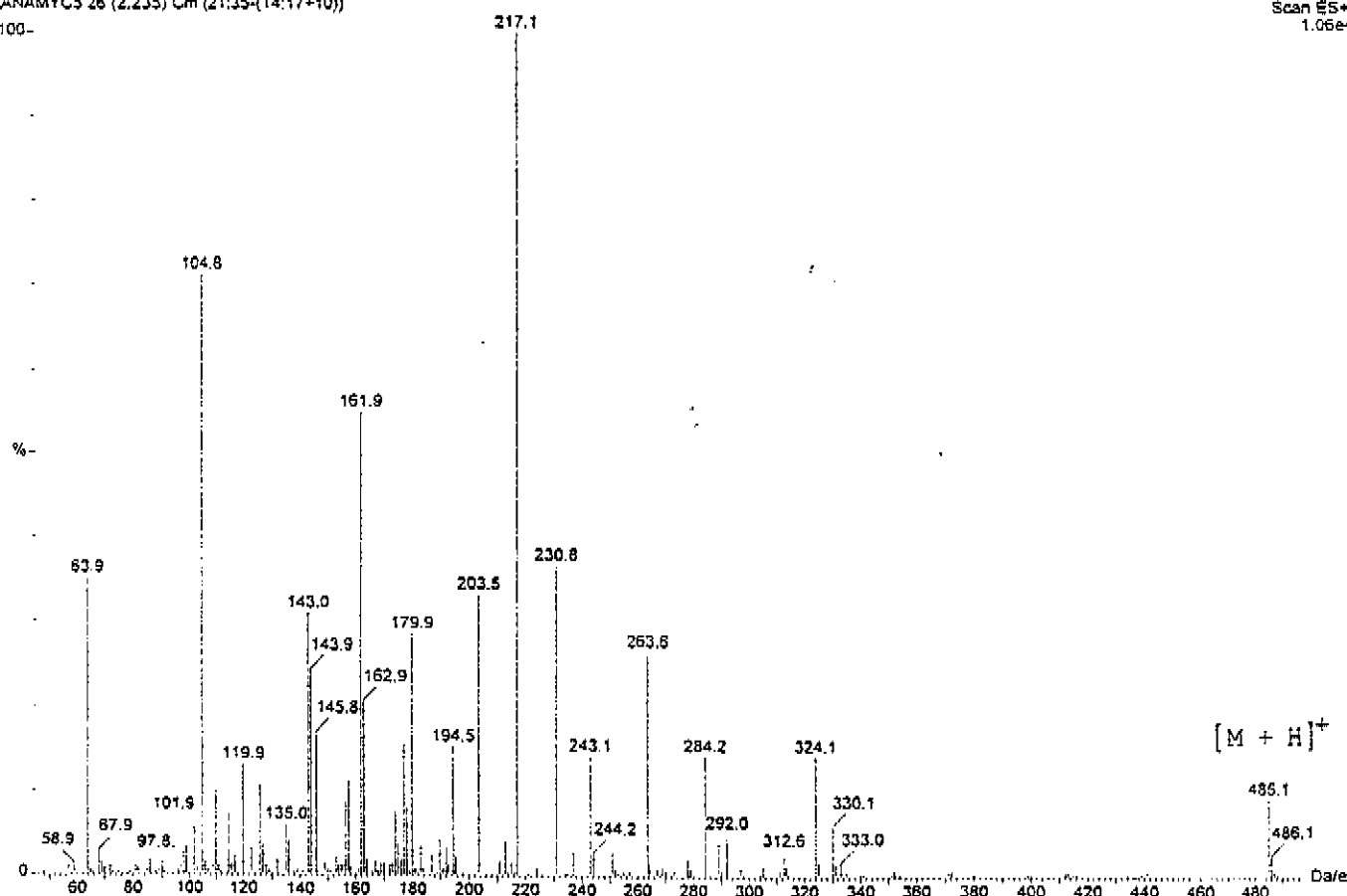


Figure 3. Positive-ion electrospray mass spectrum of kanamycin sulfate Control No 197211.

UV-spectrum

A UV-spectrum in water was recorded on a Varian Cary 5 spectrophotometer. No maximum above 190 nm was observed, the absorbance was calculated on the slope at 200 nm.

$A_{1\%}^{1\text{cm}} = 12$ at 200 nm (n=1).

The calculation was performed on the "as is" basis.

Thin-layer chromatography

For the identity of kanamycin monosulfate see results under Purity/Thin-layer chromatography.

Sulfate

For the identity and estimation of sulfate see under Purity/Ion Chromatography.

Assay

Microbiological assay

See results from collaborating laboratories.

Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 4.1 % (w/w) was observed (n=3, RSD=3.1 %). The substance was analyzed immediately after opening of the ampoule, but as the substance is extremely hygroscopic it readily picks up moisture before the analysis starts. The relative humidity at the analysis occasion was 25 %.

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.

Sample weight: 2 mg.

Heating program: 5 °C/min from 20–105 °C and then holding 105 °C for 180 minutes.

Melting point: About 270 °C with decomposition.

Water

It was not possible to determine the content of water by Karl Fischer titration due to the high hygroscopicity and the small quantity of sample available.

Organic volatile compounds

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them were estimated to be <100 ppm.

The content of organic solvents was tested by gas chromatography with the following conditions:

Instrument: Hewlett Packard 6890

Column: HP-5 (30 m x 0.53 mm, 2.65 µm film)

Carrier gas: Helium (8 ml/min)

Detector: FID

Injection volume: 2 µl

Injector temperature: 200 °C

Detector temperature: 250 °C
Temperature program: 40 °C for 10 minutes, 40 °C/min and holding 240 °C for 5 minutes.
Sample: 20 mg were shaken with 1 ml of benzylalcohol and another 20 mg were shaken with 1 ml of Milli Q purified water each for about five minutes. The sample shaken with benzylalcohol was filtered.

Purity

Thin-layer chromatography

Two secondary spots were detected. Their total amount was estimated to be about 2.1 %. One of the spots was identified as kanamycin B sulfate and roughly estimated to be 0.1 %. The following thin-layer chromatographic system according to *The International Pharmacopoeia*, Third Edition, Volume 4 was used.

Thin-layer: Silica gel (Merck) TLC.
Eluent: 7 % potassium dihydrogen phosphate.
Sample: 100 µg of kanamycin monosulfate dissolved in water were applied.
Visualization: Visualization in day-light after spraying with naphthalene-1,3-diol/ethanol and sulfuric acid, as well as scanning at 400 nm with a CAMAG TLC Scanner 3.

$R_f(\text{kanamycin sulfate})=0.2$
 $R_f(\text{kanamycin B sulfate})=0.1$

The detection limit of the system was about 0.1 µg (0.1 %) both after visual inspection and when scanning at 400 nm.

The spot of kanamycin monosulfate ICRS Control No 197211 corresponds in position and appearance with that of the EPCRS batch 2 and USPRS lot I of kanamycin sulfate.

The EPCRS contained two secondary spots. Their total amount was estimated to about 5.7 %. One of the spots was identified as kanamycin B sulfate and roughly estimated to be 0.5 %. The USPRS contained two secondary spots. Their total amount was estimated to about 5.2 %. One of the spots was identified as kanamycin B sulfate and roughly estimated to be 0.8 %.

High performance liquid chromatography

As kanamycin sulfate has practically no UV-absorbance other detection principles had to be chosen. Pre-column derivatization using 2,4-dinitrofluorobenzene as denoted in the monograph for Tobramycin of the USP XXII, was found satisfactory for the detection of kanamycin. The total amount of impurities estimated by peak area normalization was about 6.7 % (n=24, RSD=0.13 % for the main peak, RSD=2 % calculated on the 2.4 % impurity level).

As can be seen in Figure 4 thirteen impurities above the limit of quantification were found. One of them was identified as kanamycin B sulfate (eluting at 30 minutes). It was estimated to 0.4 % against an external standard. All peaks eluting before 23 minutes are blank peaks originating from the derivatization agents.

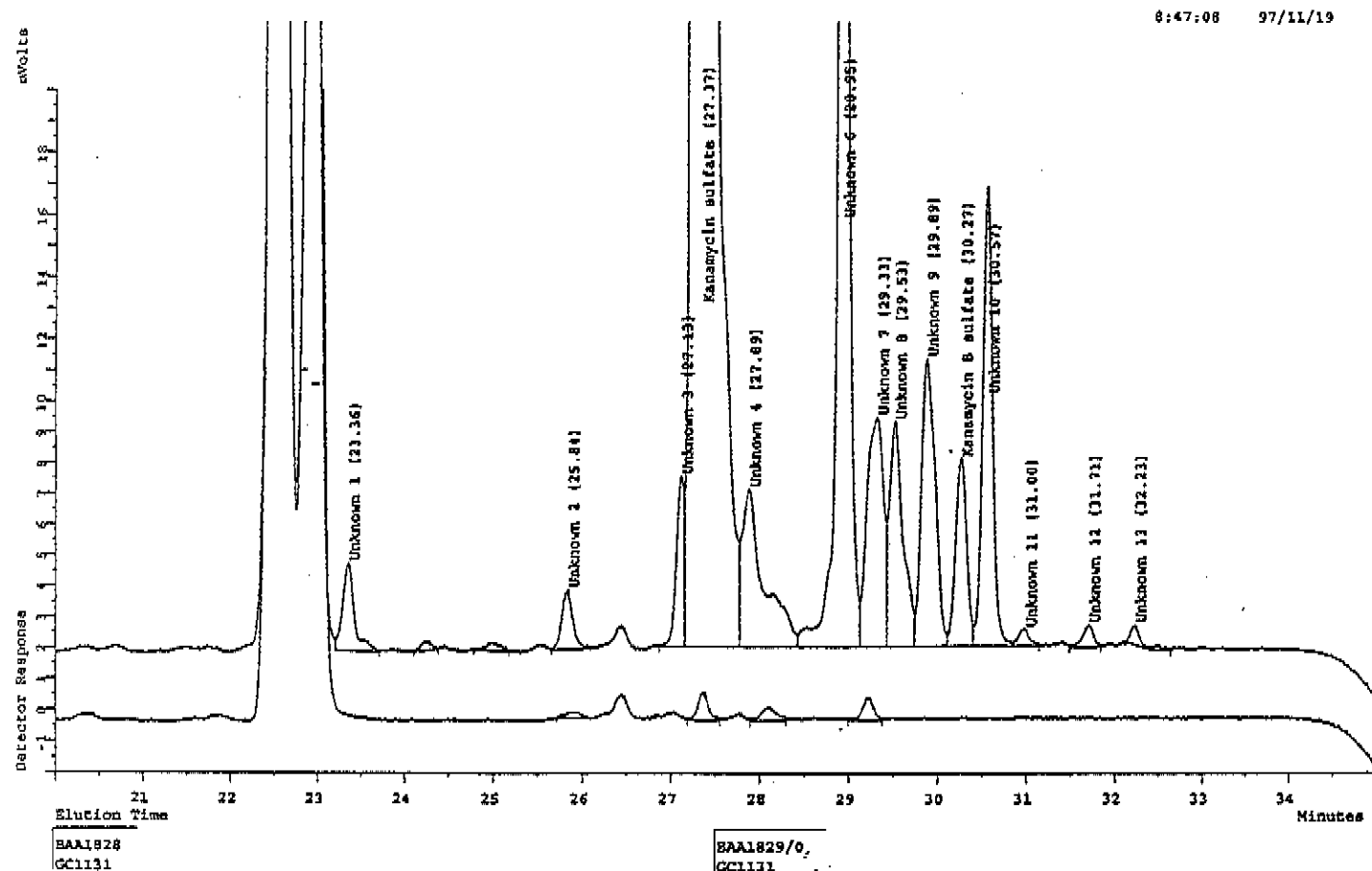


Figure 4. Chromatogram of kanamycin monosulfate Control No 197211 as 2,4-dinitro-fluorobenzene derivative monitored at 365 nm.

The following conditions were used:

Eluent:

A: Acetonitrile

B: 2.0 g of tris(hydroxymethyl)aminomethane was dissolved in water then 10.0 ml 1 M sulfuric acid was added and diluted with water to 1000 ml.

Gradient:	% A	% B	Time, minutes	Type
	30	70	0-10	isocratic
	30→80	70→20	10-30	linear
	80→30	20→70	30-31	linear
	30	70	31-45	isocratic equilibration
Column:	Brownlee Labs spheri 5 RP-18 at 40 °C, 4.6 x 250 mm, 5 µm particles.			
Detector:	Waters Lambda-Max model 481 operated at 365 nm.			
Pump:	Waters 600E operated at a flow rate of 1.0 ml/min.			
Injector:	Carnegie CMA 200 operated at 8 °C.			
Integrator:	PeakPro (Beckman)			
Sample:	Kanamycin sulfate was derivatized (see below). Sample concentration was about 0.15 mg/ml. 20 µl corresponding to 3 µg were injected.			

Derivatization:

2,4-Dinitrofluorobenzene reagent: 10 mg 2,4-dinitrofluorobenzene per ml of ethanol.

Tris(hydroxymethyl)aminomethane reagent: 20 ml of a solution containing 15 mg tris(hydroxymethyl)aminomethane per ml of water were transferred into a 100-ml volumetric flask and diluted to volume with dimethylsulfoxide. This reagent was used within 4 hours.

Purity determination: 1.50 ml of a sample solution containing 2.50 mg/ml water, were transferred into a 25-ml volumetric flask and 7 ml of the 2,4-dinitrofluorobenzene reagent and 7 ml of the tris(hydroxymethyl)aminomethane reagent were added. The solution was heated for 60 minutes in a water bath at 60 °C. The flask was left at room temperature for 10 minutes to cool before dilution to volume with acetonitrile.

N.B. As cooling occurs on mixing with acetonitrile, it is advisable to add the acetonitrile slowly, permitting the mixture to reach room temperature, before the final dilution.

Stability in the eluent: A sample was stored for 24 hours at 8 °C in the dark. No signs of change in the derivatized sample were observed.

Limit of detection: 0.5 ng (0.02 %) at 365 nm

Limit of quantification: 2 ng (0.06 %) at 365 nm

Ion chromatography

The ICRS was screened for the following anions: chlorides, bromides, nitrates, phosphates and sulfates. Less than 10 ppm of bromides, less than 100 ppm of nitrates and less than 500 ppm of phosphates were found. Chlorides were found and estimated at 500 ppm. Sulfates were found and roughly estimated to be 16.2 % (theoretical value 16.0 %).

The following conditions were used:

Eluent 1:	Sodium carbonate/sodium bicarbonate 1.8 mM/1.7 mM
Eluent 2:	Sodium bicarbonate (for the determination of chloride) 2.5 mM
Column:	Dionex HPIC AS4A
Suppressor:	ASRS-1
Detector:	Dionex 2000i, conductivity
Range:	3 μ S (for determination of sulfate 300 μ S)
Pump:	Dionex 2000i
Injector:	Beckman System Gold Autoinjector 507
Integrator:	PeakPro (Beckman)
Sample:	Kanamycin sulfate was dissolved in the eluent at a concentration of 1.0 mg/ml in water. 50 μ l corresponding to 50 μ g were injected.

Ion	Chlorides	Bromides	Nitrates	Phosphates	Sulfates
Limit of detection, ppm in the sample:	10	10	30	100	30
Limit of quantification, ppm in the sample:	50	50	100	500	100

Data given by collaborating laboratories

NIBSC

Description:	The first International Standard for Kanamycin (Established in 1986)
Microbiological assay, determined in a collaborative study:	10345 International Units per ampoule.
Moisture content:	< 0.2 % in the closed ampoule.
Stability:	Accelerated degradation studies on the sealed ampoules at 56 °C for 20 months showed no loss in potency.
Hygroscopicity:	The ampoule contents are extremely hygroscopic.

Stability

Stability studies were not performed as this substance was not suspected to degrade easily when stored at -20 °C. Regular re-examinations of the ICRS when stored in the dry state will be performed.

Hygroscopicity

The ampoule contents are extremely hygroscopic. When stored for 7 hours at 98 % relative humidity the substance gained 30 % in weight. Results are given in Figure 5. After 30 minutes it gained 16 % in weight. At continued storage for 24 hours to one week at 98 % relative humidity, the content of water decreases to 3-7 %.

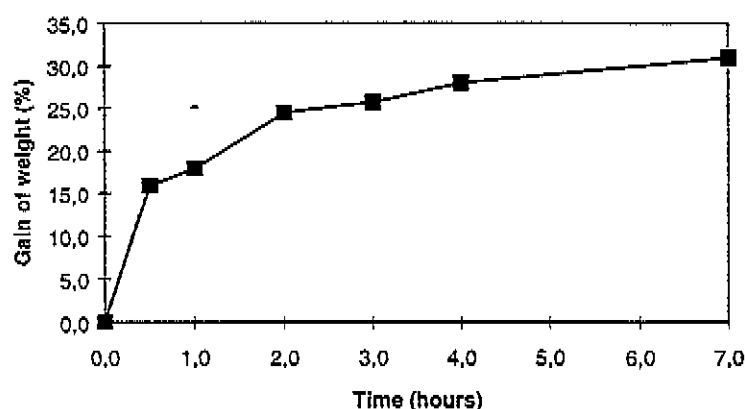


Figure 5. *Kanamycin monosulfate Control No 197211 stored at 98 % relative humidity.*

As the substance is extremely hygroscopic it is advisable to let it equilibrate with the surrounding atmosphere before weighing it.

Conclusion

Kanamycin monosulfate, Control No 197211, can be considered suitable as International Chemical Reference Substance for the intended purpose. As this is a biological substance it is difficult to obtain a better quality. This ICRS is purer than other official reference materials.

Phenacetin

WHO Melting Point Reference Substance

Melting temperature 136 °C

Control No 297172

Analytical Report

Intended use

The stock of the current batch of the WHO Melting Point Reference Substance for phenacetin, Control No 192172, is depleted and has to be replaced.

The WHO Melting Point Reference Substance for phenacetin is supplied primarily for calibration of different instruments and methods used for determination of melting temperatures against the method of *The International Pharmacopoeia*, Third Edition, Volume 1.

Material

About 4000 g of the sample (manufacturers batch no 207372) were received at the WHO Centre in April 1997. The material is being stored in tightly closed containers at + 20 °C, protected from light.

Analytical data

Description

A white powder.

Evidence of chemical structure

Infrared spectrum

The spectrum is concordant with the spectrum of the previous lot of the International Chemical Reference Substance (ICRS) of phenacetin with Control No 192172. An infrared spectrum is given in Figure 1 (No W297172T).

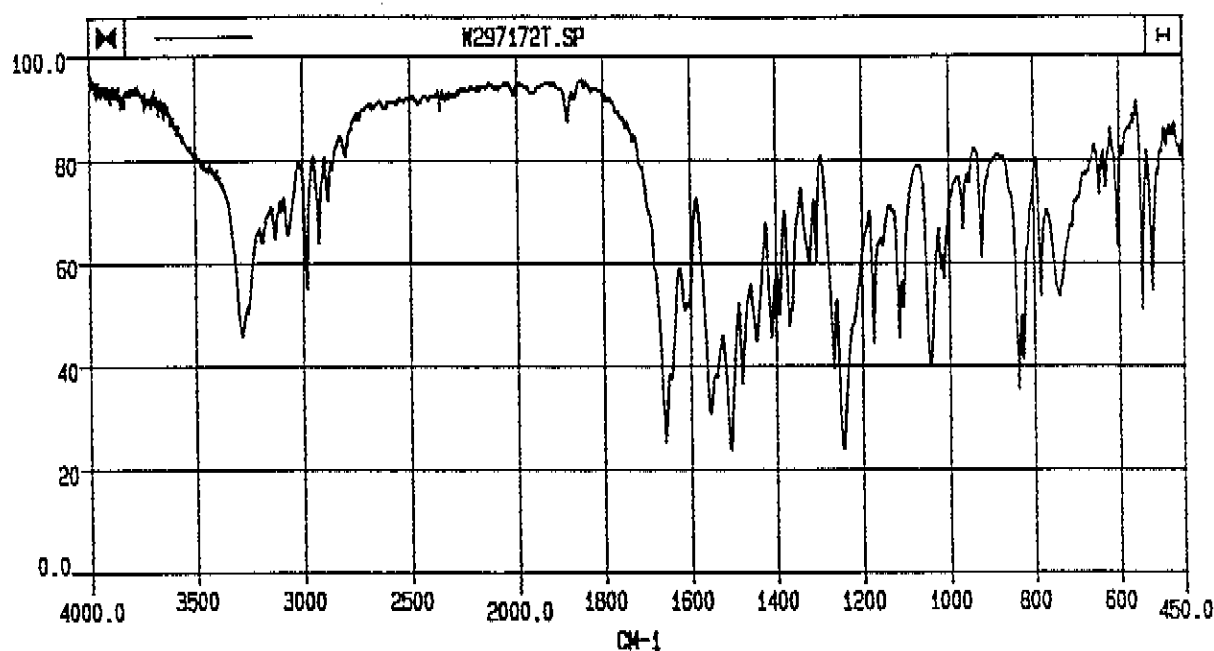


Figure 1. IR-spectrum of 1.0 mg of phenacetin Control No 297172 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

Purity

Assigned melting point: 136 °C

Based on results from a collaborative study. See under Collaborative study at the end of this Appendix.

Water

< 0.1 % (n=2) determined by Karl Fischer titration.

High performance liquid chromatography

One impurity was found. It was estimated to be 0.06 % (n=6, RSD=2.6 % when calculated on the 0.06 % impurity level, RSD< 0.01 % for the main peak). A chromatogram is given in Figure 2.

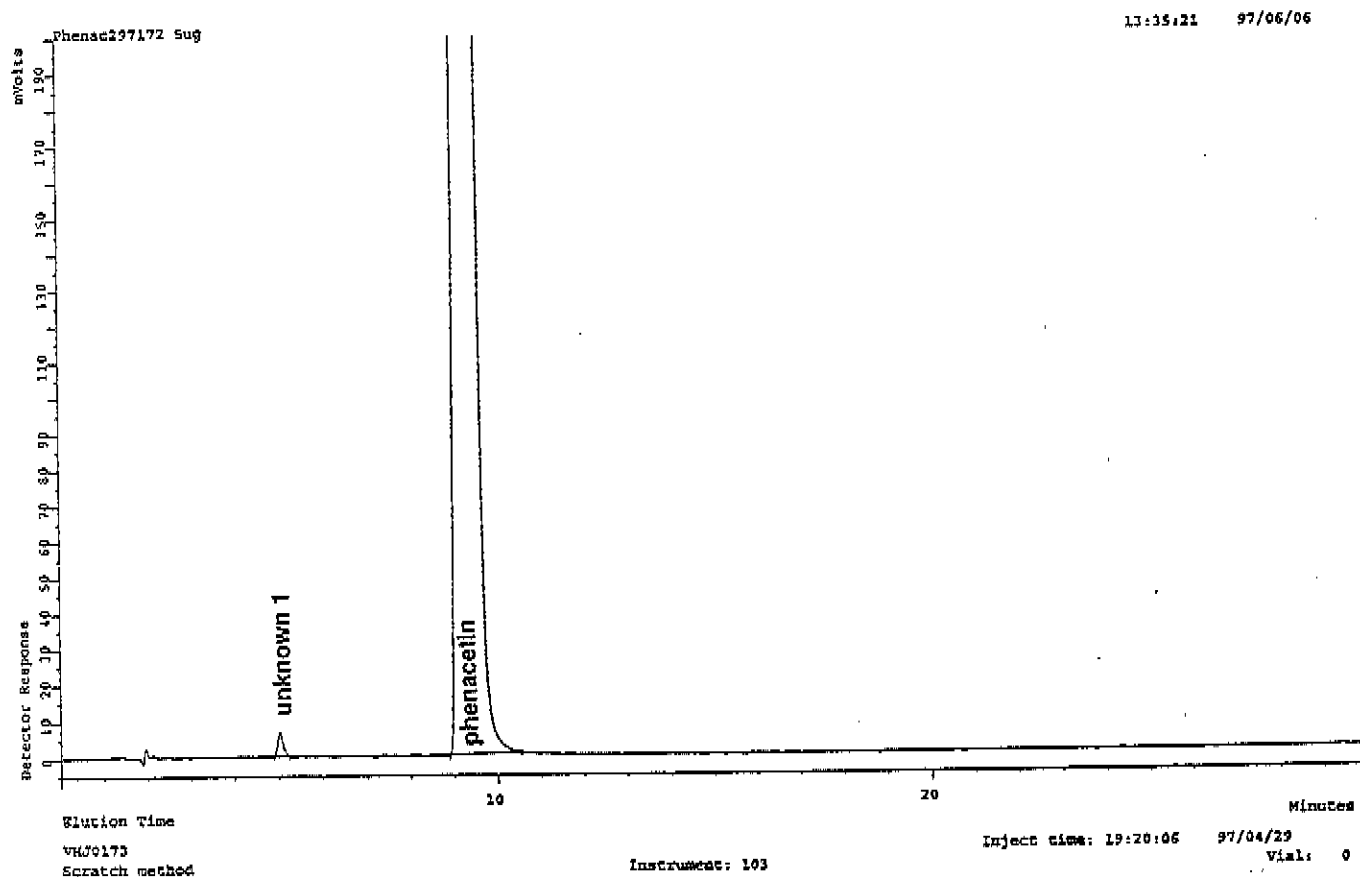


Figure 2. Chromatogram of phenacetin Control No 297172 monitored at 247 nm.

The following conditions were used:

Eluent:	Acetonitrile:0.05 M phosphate buffer pH 3.0 (23:77)
Column:	Kromasil C18, 5 μ m, 4.6 x150 mm
Detector:	Gynkotek UVD 340S operated at 247 nm.
Pump:	Gynkotek M480 operated at a flow rate of 1.0 ml/min.
Injector:	Gynkotek Gina 50 operated at 8 °C.
Integrator:	PeakPro (Beckman)
Sample:	Phenacetin was dissolved in the eluent at a concentration of 1.0 mg/ml. 5 μ l corresponding to 5 μ g were injected.
Stability in the eluent:	A sample was stored in the eluent for about ten hours in the dark at +8 °C. No signs of degradation were observed. When the sample was stored in daylight for one week at + 20 °C a degradation of less than 0.06 % was observed.
Limit of detection:	1 ng (0.02 %) at 247 nm
Limit of quantification:	3 ng (0.06 %) at 247 nm

In the previous batch ICRS 192172 two impurities were found and estimated to be about 0.2 %.

Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of the main peak and the peak of impurity 1 were similar with UV-maxima at 247 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities. A UV-spectrum of the main peak recorded in the eluent is given in Figure 3.

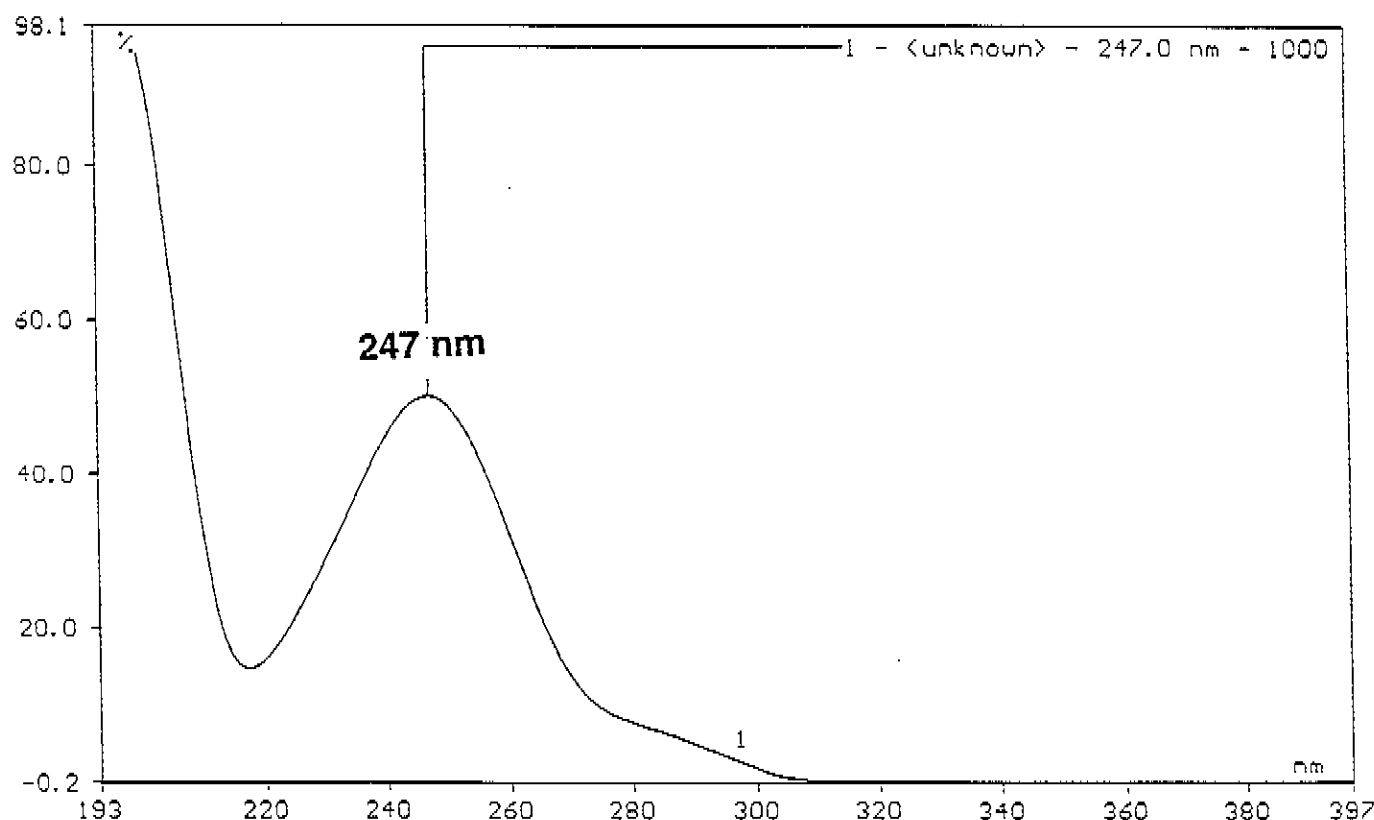


Figure 3. *UV-spectrum of phenacetin recorded in the eluent.*

Capillary electrophoresis

No impurities above the limit of quantification (0.1 %) were found.

An electropherogram is given in Figure 4.

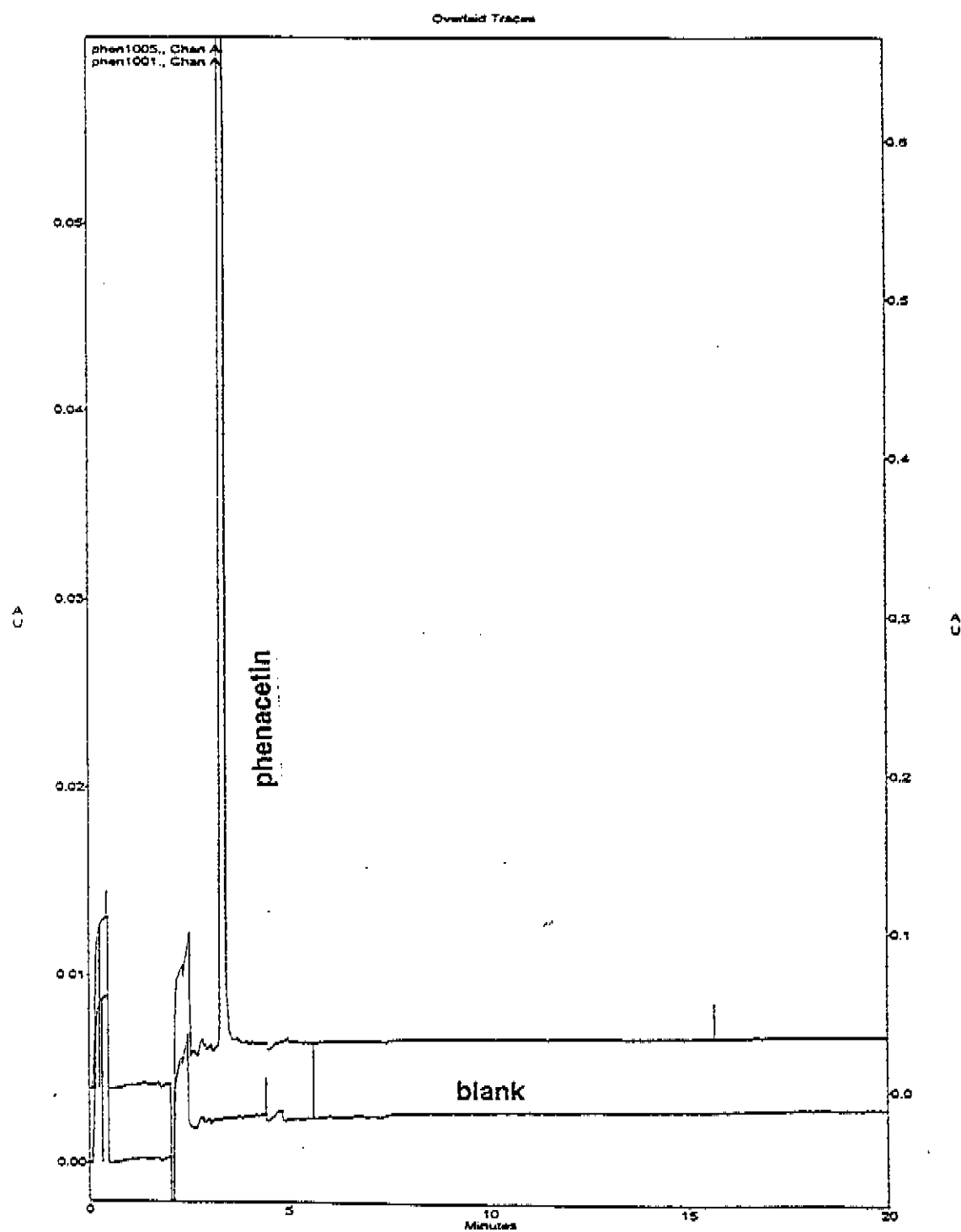


Figure 4. Electropherogram of phenacetin Control No 297172 monitored at 247 nm.

Eluent:	11.7 mM borate, 8.3 mM phosphate and 50 mM SDS at pH 9.20
Capillary:	Fused silica (total length 67 cm, length to detector 60 cm, ID: 75 μ m)
Detector:	Beckman P/ACE Diode Array Detector operated at 247 nm and 200 nm.
CE Instrument:	Beckman P/ACE system 5510 operated with an applied voltage of 30 kV for 20 minutes.
Run temperature:	50 °C.
Injection:	10 seconds pressure injection.
Integrator:	Beckman P/ACE Station Software.
Sample:	Phenacetin was dissolved in water at a concentration of 20.0 mg/ml in methanol and then diluted with water to a concentration of 2.0 mg/ml (fresh solutions). 10 seconds high pressure injection corresponds to 118 ng injected sample.
Limit of detection:	0.03 % at 247 nm
Limit of quantification:	0.10 % at 247 nm

Differential scanning calorimetry

The purity was estimated to about 99.99 mol % (n=10, RSD=0.007 %) determined by differential scanning calorimetry. The determination was performed on 2 mg using a heating rate of 2 °C per minute.

Melting temperature (T _M):	134.4 °C (n=10, RSD=0.09 %)
Onset:	134.3 °C (n=10, RSD=0.07 %)
Instrument:	Perkin Elmer DSC7 Differential Scanning Calorimeter

No signs of polymorphic forms were found, when the substance was heated from 40 °C to 200 °C.

The previous batch ICRS 197172 showed the same result.

Data given by the manufacturer

Identification:	Conforms.
Assay:	99.3 %
Chloroacetanilide:	within limits
p-Phenetidine:	within limits
Heavy metals:	within limits
Sulphated ash:	0.0 %
Loss on drying:	0.0 %

Collaborative study

The melting point was assigned after a collaborative study including four laboratories. The melting point was determined according to *The International Pharmacopoeia*, Third Edition, Volume 1, as a capillary melting point determination.

Participating laboratories:

Lab 1: Björn Egil Olsen
Norsk Medicinaldepot AS
Sven Oftedalsvej 10
PO Box 100
N 0518 OSLO
NORWAY

Lab 2: Camilla Alvesson
Mettler Toledo AB
Hängpilsgatan 56
42677 Västra Frölunda
GÖTEBORG
SWEDEN

Lab 3: Dr Ulrich Rose
Service de la Qualité du Médicament
European Pharmacopoeia Laboratory
B.P. 907
F-67029 Strasbourg Cedex 1
FRANCE

Lab 4: WHO Collaborating Centre for Chemical Reference Substances
STOCKHOLM
SWEDEN

RESULTS

Method	Lab 1	Lab 2	Lab 3	Lab 4	Mean
Capillary melting point, °C	136.1	135.7	135.9	136.2	136.0
n	6	6	6	6	4
RSD, %	0.06	0.04	0.07	0.09	0.16

Assigned melting point: 136 °C

Stability

No special stability studies were performed as it was considered that this substance, based on the experience of the stability of the previous lot, was stable and showed no signs of degradation when stored for 34 years at + 20 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

Conclusion

Phenacetin, Control No 297172, can be considered suitable as WHO Melting Point Reference Substance for the intended purpose with the melting point set to 136 °C.

Piperazine adipate

Control No 197212

Analytical Report

Intended use

The monograph for Piperazine adipate tablets in *The International Pharmacopoeia*, Third Edition, Volume 4, requires a reference substance of piperazine adipate to be used in the thin-layer chromatographic test for identity.

Material

About 10 g of the sample (manufacturers batch NOENR4786) were received at the WHO Centre in June 1996. The material is being stored in tightly closed containers at + 5 °C, protected from light.

This ICRS is of the same origin as that of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1.

Analytical data

Description

A white powder.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum is given in Figure 1 (W197212). The spectrum is concordant with the spectrum of the United States Pharmacopoeia Reference Standard (USPRS) lot F of piperazine adipate.

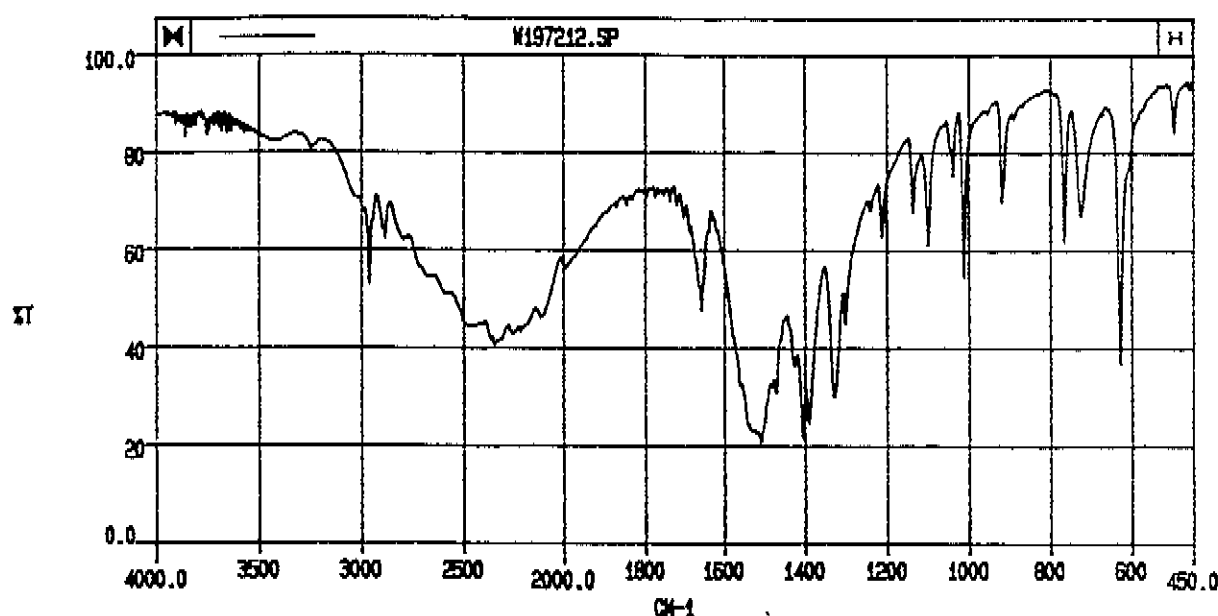


Figure 1. IR-spectrum of 0.8 mg of piperazine adipate Control No 197212 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of piperazine adipate was recorded by particle beam electron ionization (PB-EI) in the positive ion mode. The spectrum, which is given in Figure 2, is concordant with the spectrum of piperazine adipate published in the National Institute of Standards and Technology (NIST) library.

Eluent:	Methanol:Water (50:50)
Column:	Direct inlet
Pump:	Hewlett Packard 1050 operated at a flow rate of 0.5 ml/min
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Electron energy 70 V
	Source temperature 250 °C
Sample:	Piperazine adipate was dissolved in water at a concentration of 0.1 mg/ml. 10 µl corresponding to 1 µg were injected.

Piperazine adipate ICRS 197212 EI+ 250 C
PIPERAA1 54 (4.815) Cm (52:57-(48:50+63:84))

Scan EI+
5.62e4

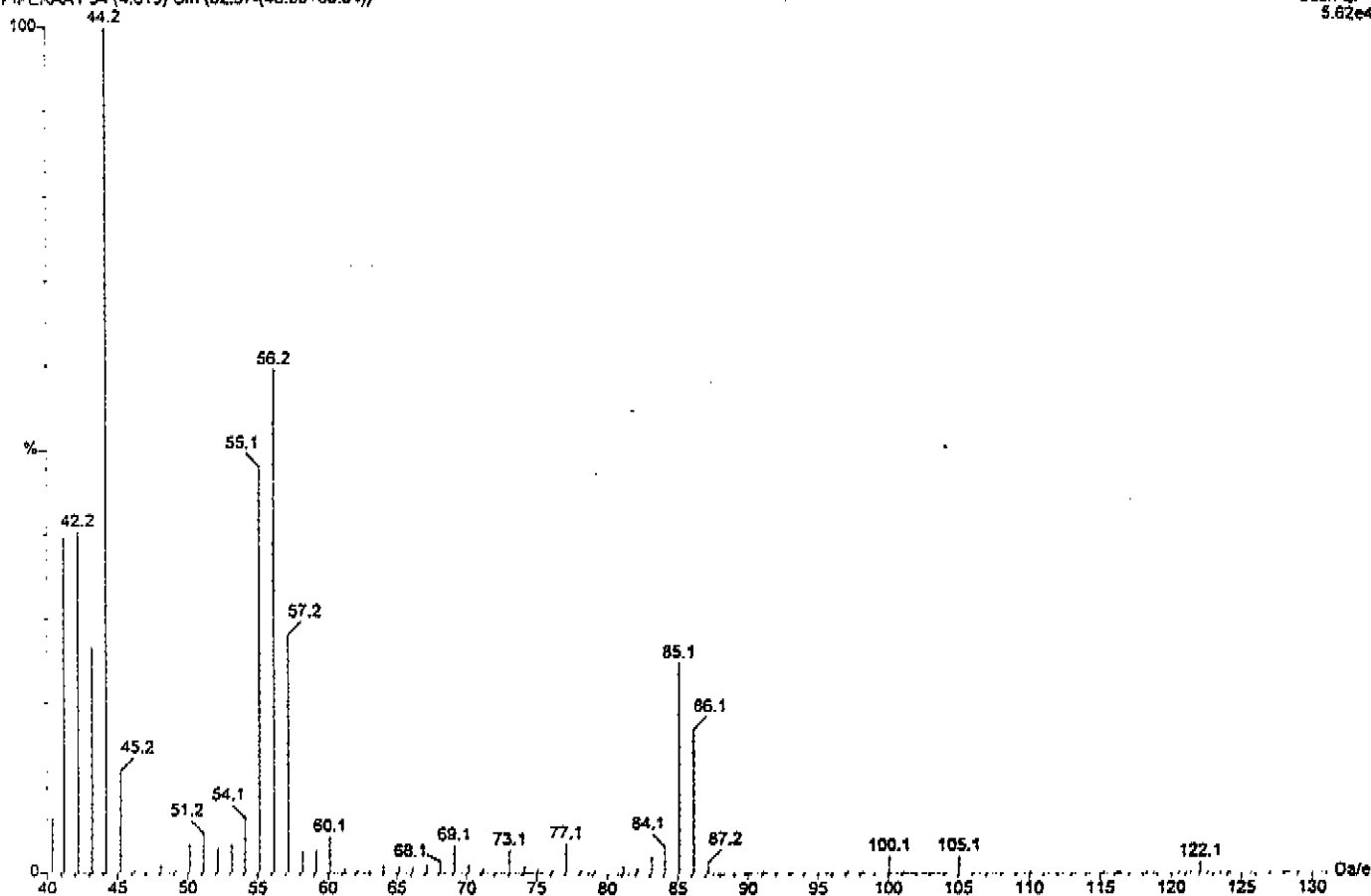


Figure 2. Positive-ion electron ionization mass spectrum of piperazine adipate Control No 197212.

UV-spectrum

Piperazine adipate has practically no UV absorbance except at wavelengths below 210 nm.

A UV-spectrum in water was recorded on a Varian Cary 5 spectrophotometer.

A UV-maximum was observed at 195 nm. $A_{1\text{cm}}^{1\%}$ was about 46 (n=1).

Thin-layer chromatography

For the identity of piperazine adipate see results under Purity/Thin-layer chromatography.

Assay

See results from collaborating laboratories.

Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of < 0.1 % (w/w) was observed (n=6).

Instrument:	Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight:	10 mg.
Heating program:	5 °C/min from 20–105 °C and then holding 105 °C for 360 minutes.
Melting point:	250 °C with decomposition.

Water

0.1 % (n=2), determined by Karl Fischer titration.

Organic volatile compounds

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them were estimated to be < 100 ppm.

The content of organic solvents was tested by gas chromatography with the following conditions:

Instrument:	Hewlett Packard 6890
Column:	HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas:	Helium (8 ml/min)
Detector:	FID
Injection volume:	2 µl
Injector temperature:	200 °C
Detector temperature:	250 °C
Temperature program:	40 °C for 10 minutes, 40 °C/min and holding 240 °C for 5 minutes.
Sample:	20 mg were shaken with 1 ml of benzyl alcohol and another 20 mg were shaken with 1 ml of Milli Q purified water each for about five minutes. The sample shaken with benzyl alcohol was filtered.

PurityThin-layer chromatography

No secondary spots were detected, which means < 0.25 %. The following thin-layer chromatographic system according to *The International Pharmacopoeia*, Third Edition, Volume 4, was used.

Thin-layer:	Silica gel G (Merck) TLC
Eluent:	Ammonia conc.:acetone (20:80)
Sample:	500 µg of piperazine adipate dissolved in ethanol:ammonia conc. (2:3) were applied.
Visualization:	Visualization in day-light after spraying with triketohydrindenehydrate (3 mg/ml) in glacial acetic acid/1-butanol (3:100) and then with triketohydrindenehydrate (1.5 mg/ml) in ethanol and drying at 105 °C. Thereafter spraying with iodine (0.05 mol/l).

$R_f(\text{piperazine adipate})=0.1$

$R_f(\text{ethylenediamine})=0.4$

$R_f(\text{triethylenediamine})=0.2$

$R_f(\text{adipic acid})=0.04$

$R_f(\text{piperazine})=0.08$

The detection limit of the system was about 1.25 µg (0.25 %) when examined visually.

The spot of piperazine adipate corresponds in position and appearance with that of the USPRS lot F of piperazine adipate.

Ion chromatography

The ICRS was screened for the following anions: chlorides, bromides, nitrates, phosphates and sulfates. Less than 300 ppm of chlorides, less than 10 ppm of bromides, less than 100 ppm of phosphates and less than 30 ppm of nitrates were found. Sulfates co-eluted with adipate and could not be determined.

The following conditions were used:

Eluent 1:	Sodium carbonate/sodium bicarbonate 1.8 mM/ 1.7 mM
Eluent 2:	Sodium bicarbonate (for the determination of chloride) 2.5 mM
Column:	Dionex HPIC AS4A
Suppressor:	ASRS-1
Detector:	Dionex 2000i, conductivity
Range:	3 µS
Pump:	Dionex 2000i
Injector:	Beckman System Gold Autoinjector 507
Integrator:	PeakPro (Beckman)
Sample:	Piperazine adipate was dissolved in the eluent at a concentration of 1.0 mg/ml in water. 50 µl corresponding to 50 µg were injected.

Ion	Chlorides	Bromides	Nitrates	Phosphates	Sulfates
Limit of detection, ppm in the sample:	10	10	30	100	30
Limit of quantification, ppm in the sample:	50	50	100	500	100

Data given by collaborating laboratories

European Pharmacopoeia lab.

EPCRS batch 1 is from the same batch as ICRS 197212.

Appearance:	Satisfactory
Water content:	0.1 %
Identity, IR:	Conforms.
Identity, melting point:	248 °C
Identity, melting point:	151 °C (as piperazine)
Related substances:	0.3 %
Assay:	100.22 %

Stability

Stability studies were not performed as this substance was not suspected to degrade easily according to WHO/PHARM/86.529. Regular re-examinations of the ICRS when stored in the dry state will be performed.

Conclusion

Piperazine adipate, Control No 197212, can be considered suitable as International Chemical Reference Substance for the intended purpose.

Piperazine citrate

Control No 197213

Analytical Report

Intended use

The monograph for Piperazine citrate tablets in *The International Pharmacopoeia*, Third Edition, Volume 4, requires a reference substance of piperazine citrate to be used in the thin-layer chromatographic test for identity.

Material

About 10 g of the sample (manufacturers batch NOENR4787) were received at the WHO Centre in June 1996. The material is being stored in tightly closed containers at + 5 °C, protected from light.

This ICRS is of the same origin as that of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1.

Analytical data

Description

A white granular powder.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum is given in Figure 1 (W197213). The spectrum is concordant with the spectrum of the United States Pharmacopoeia Reference Standard (USPRS) lot F of piperazine citrate.

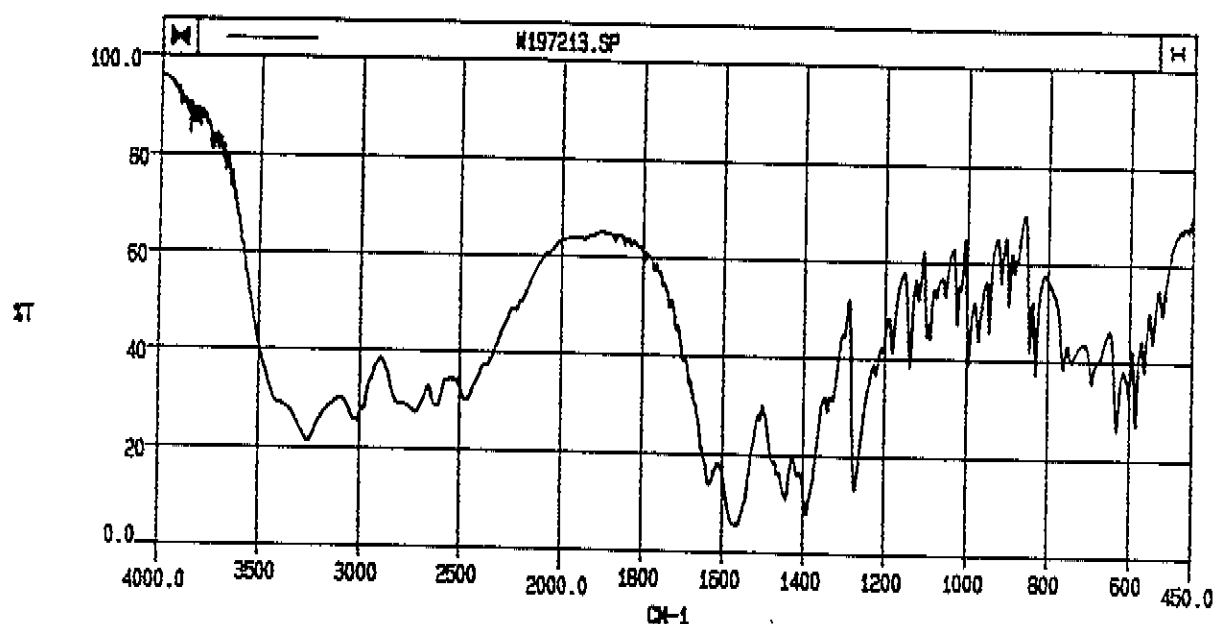


Figure 1. IR-spectrum of 1.1 mg of piperazine citrate Control No 197213 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of piperazine, given in Figure 2, was recorded by atmospheric pressure chemical ionization (APCI) in the positive ion mode. The spectrum shows an $[M + H]^+$ ion of 87.2, which supports the identity of piperazine.

Eluent:	Methanol:Water (50:50)
Column:	Direct inlet
Pump:	Hewlett Packard 1050 operated at a flow rate of 0.3 ml/min
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Cone voltage 30 V
	Source temperature 180 °C
Sample:	Piperazine citrate was dissolved in water at a concentration of 0.01 mg/ml. 20 µl corresponding to 200 ng were injected.

Piperazine citrate ICRS 197213 APci+ 180 C Direct inlet
PIPERAC9 86 (7.335) Cm (84:97-(106:113+71:78))

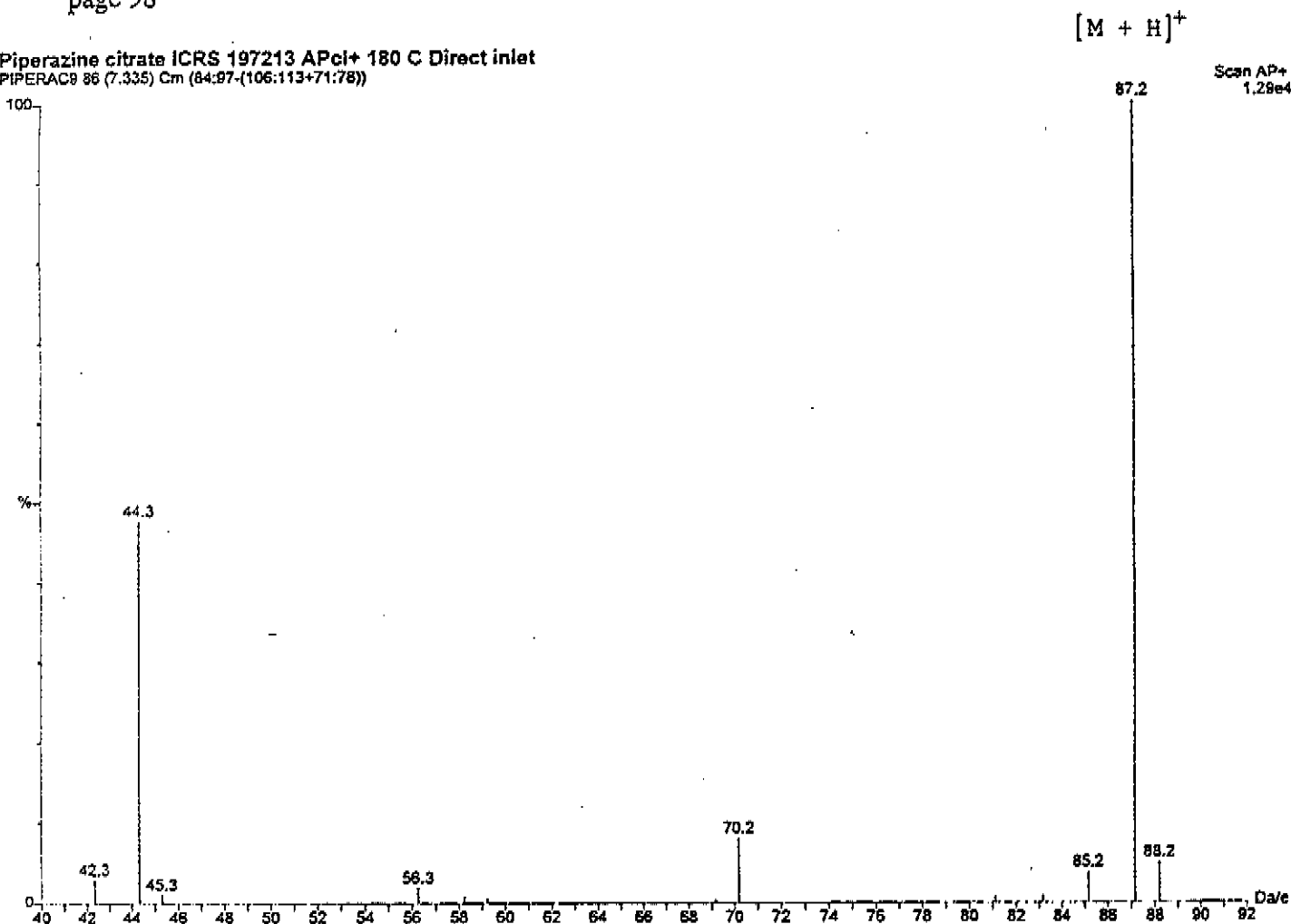


Figure 2. Positive-ion atmospheric pressure chemical ionization mass spectrum of piperazine citrate Control No 197213.

UV-spectrum

Piperazine citrate has practically no UV absorbance except at wavelengths below 210 nm.

A UV-spectrum in water was recorded on a Varian Cary 5 spectrophotometer.

A UV-maximum was observed at 191 nm. $A_{1\%}^{1\text{cm}}$ was about 30 (n=1).

Thin-layer chromatography

For the identity of piperazine citrate see results under Purity/Thin-layer chromatography.

Citrate

The identity of citrate was confirmed according to *The International Pharmacopoeia*, Third Edition, Volume 1 by the general identification test for citrate.

Assay

See results from collaborating laboratories.

Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 11.1 % (w/w) was observed (n=6, RSD=0.35%).

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 10 mg.
Heating program: 5 °C/min from 20–105 °C and then holding 105 °C for 360 minutes.
Melting point: 183–187 °C with decomposition.

Water

11.3 % (n=2), determined by Karl Fischer titration.

Organic volatile compounds

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them were estimated to be < 100 ppm.

The content of organic solvents was tested by gas chromatography with the following conditions:

Instrument: Hewlett Packard 6890
Column: HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas: Helium (8 ml/min)
Detector: FID
Injection volume: 2 µl
Injector temperature: 200 °C
Detector temperature: 250 °C
Temperature program: 40 °C for 10 minutes, 40 °C/min and holding 240 °C for 5 minutes.
Sample: 20 mg were shaken with 1 ml of benzyl alcohol and another 20 mg were shaken with 1 ml of Milli Q purified water each for about five minutes. The sample shaken with benzyl alcohol was filtered.

Purity

Thin-layer chromatography

No secondary spots were detected, which means < 0.25 %. The following thin-layer chromatographic system according to *The International Pharmacopoei*, Third Edition, Volume 4, was used.

Thin-layer: Silica gel G (Merck) TLC
Eluent: Ammonia conc.:acetone (20:80)
Sample: 500 µg of piperazine citrate dissolved in ethanol:ammonia conc. (2:3) were applied.

Visualization: Visualization in day-light after spraying with triketohydrindenehydrate (3 mg/ml) in glacial acetic acid/1-butanol (3:100) and then with triketohydrindenehydrate (1.5 mg/ml) in ethanol and drying at 105 °C. Thereafter spraying with iodine (0.05 mol/l).

R_f (piperazine citrate)=0.06

R_f (ethylenediamine)=0.18

R_f (triethylenediamine)=0.08

R_f (piperazine)=0.06

R_f (citrate)=0.02

The detection limit of the system was about 1.25 µg (0.25 %) when examined visually.

The spot of piperazine citrate corresponds in position and appearance with that of the USPRS lot F of piperazine citrate.

Ion chromatography

The ICRS was screened for the following anions: chlorides, bromides, nitrates, phosphates and sulfates. < 200 ppm of chlorides, < 10 ppm of bromides, < 30 ppm of nitrates, < 500 ppm of sulfates and < 600 ppm of one unknown ion were found.

The following conditions were used:

Eluent 1:	Sodium carbonate/sodium bicarbonate 1.8 mM/ 1.7 mM
Eluent 2:	Sodium bicarbonate (for the determination of chloride) 2.5 mM
Column:	Dionex HPIC AS4A
Suppressor:	ASRS-1
Detector:	Dionex 2000i, conductivity
Range:	3 µS
Pump:	Dionex 2000i
Injector:	Beckman System Gold Autoinjector 507
Integrator:	PeakPro (Beckman)
Sample:	Piperazine citrate was dissolved in the eluent at a concentration of 1.0 mg/ml in water. 50 µl corresponding to 50 µg were injected.

Ion	Chlorides	Bromides	Nitrates	Phosphates	Sulfates
Limit of detection, ppm in the sample:	10	10	30	100	30
Limit of quantification, ppm in the sample:	50	50	100	500	100

Data given by collaborating laboratories

European Pharmacopoeia lab.

EPCRS batch 1 is from the same batch as ICRS 197213.

Appearance: Satisfactory.

Identity, IR: Conforms.

Water content: 11.3 %

Identity, melting point: 191 °C

TLC: Complies

Stability

Stability studies were not performed as this substance was not suspected to degrade easily according to WHO/PHARM/86.529. Regular re-examinations of the ICRS when stored in the dry state will be performed.

Conclusion

Piperazine citrate, Control No 197213, can be considered suitable as International Chemical Reference Substance for the intended purpose.

Streptomycin sulfate

Control No 197215

Analytical Report

Intended use

The monograph for Streptomycin sulfate powder for injections in *The International Pharmacopoeia*, Third Edition, Volume 4, requires a reference substance of streptomycin sulfate to be used in the thin-layer chromatographic test for identity.

Material

About 21 g of the sample were received at the WHO Centre in January 1998. The material is being stored in sealed ampoules at -20 °C, protected from light.

This ICRS is of the same origin as that of the 3rd International Biological Standard for streptomycin sulfate.

Analytical data

Description

A white fluffy freeze-dried sample.

Evidence of chemical structure

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W197215T). The spectrum is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1 of streptomycin sulfate as well as with the spectrum of the United States Pharmacopoeia Reference Standard (USPRS) lot H of streptomycin sulfate.

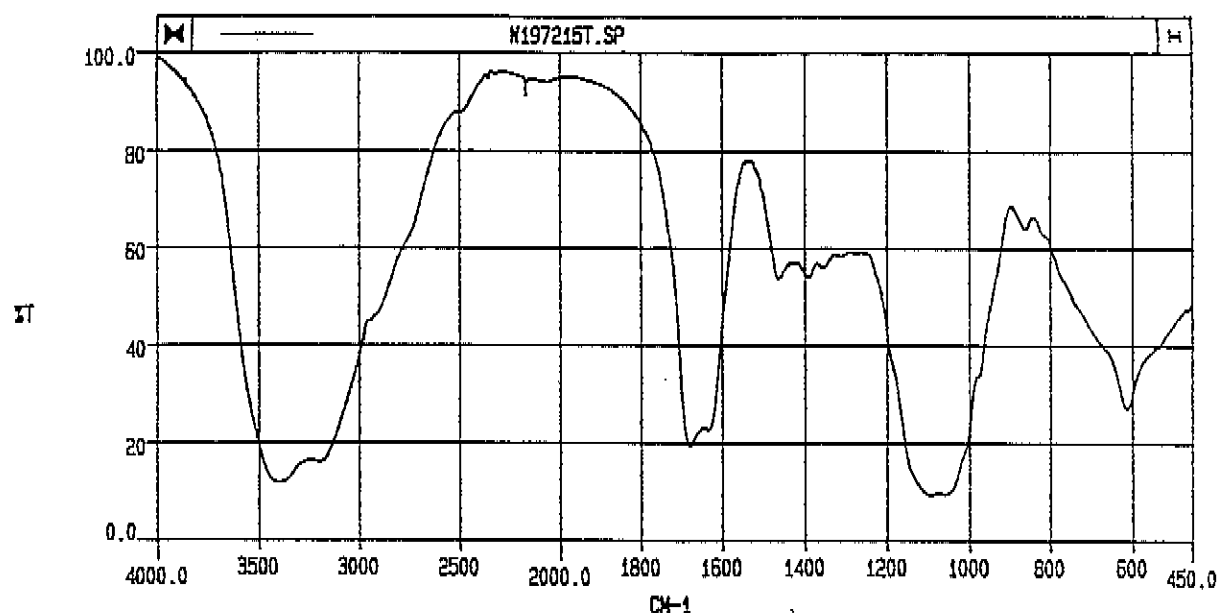


Figure 1. IR-spectrum of 1.1 mg of streptomycin sulfate Control No 197215 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer 1600 FTIR.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of streptomycin was recorded by electrospray (ESI) in the positive ion mode. The spectrum shows an $[M + H]^+$ ion of 582.0, which supports the identity of streptomycin. The spectrum, which is given in Figure 2, is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 1.

Eluent:	Acetonitrile:1 % glacial acetic acid (50:50)
Column:	Direct inlet
Pump:	Hewlett Packard 1050 operated at a flow rate of 0.2 ml/min
Detector:	Fisons Platform II (quadrupole mass spectrometer)
Operating conditions:	Cone voltage 65 V Source temperature 140 °C
Sample:	Streptomycin sulfate was dissolved in the eluent at a concentration of 0.01 mg/ml. 20 µl corresponding to 200 ng were injected.

Streptomycin sulfate ICRS 197215 ESI+ 140 C Direct inlet
STREPTO2 179 (15,240) Cm (173:184-(184:171+161:167))

Scan ES+
2.52e3

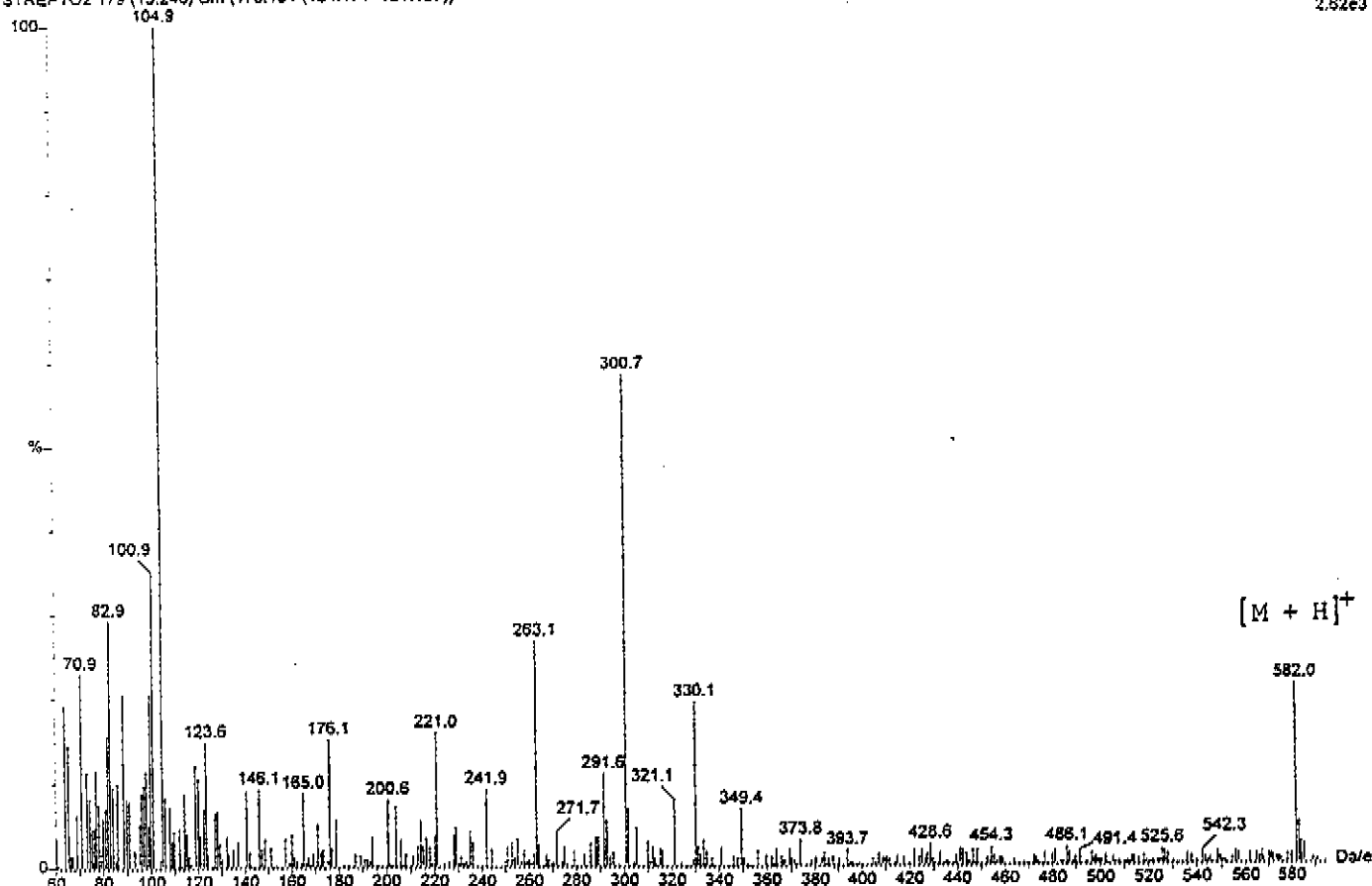


Figure 2. Positive-ion electrospray mass spectrum of streptomycin sulfate Control No 197215.

UV-spectrum

A UV-spectrum in water was recorded on a Varian Cary 5 spectrophotometer. Streptomycin sulfate has no distinct maximum but a slope increasing towards lower wavelengths was observed.

$A_{1\%}^{1\text{cm}} = 160$ at 200 nm ($n=1$).

The calculation was performed on the "as is" basis.

Thin-layer chromatography

For the identity of streptomycin sulfate see results under Purity/Thin-layer chromatography.

Sulfate

For the identity and estimation of sulfate see results under Purity/Ion Chromatography.

Assay

Microbiological assay

See results under collaborating laboratories.

Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 1.8 % (w/w) was observed (n=2, RSD=28.3 %). The substance is hygroscopic and picks up moisture immediately. This determination was performed on freshly opened ampoules. If the sample was allowed to equilibrate with the atmosphere for 24 hours at a relative humidity of 22-27 % a loss of 8.1 % (w/w) was observed (n=2, RSD=18.8 %).

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 3-5 mg.
Heating program: 5 °C/min from 20-105 °C and then holding 105 °C for 240 minutes.
Melting point: Indefinite melting range. Streptomycin sulfate becomes slightly discoloured already when heating to 105 °C.

Water

8.5 % (n=2, RSD=7.7 %) determined by Karl Fischer titration. The determination was performed on samples that had equilibrated for 24 hours with the atmosphere at a relative humidity of 22-27 %.

Organic volatile compounds

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them were estimated to be < 100 ppm.

The content of organic solvents was tested by gas chromatography with the following conditions:

Instrument: Hewlett Packard 6890
Column: HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas: Helium (8 ml/min)
Detector: FID
Injection volume: 2 µl
Injector temperature: 200 °C
Detector temperature: 250 °C
Temperature program: 40 °C for 10 minutes, 40 °C/min and holding 240 °C for 5 minutes.
Sample: 20 mg were shaken with 1 ml of benzyl alcohol and another 20 mg were shaken with 1 ml of Milli Q purified water each for about five minutes. The sample shaken with benzyl alcohol was filtered.

Purity

Thin-layer chromatography

One secondary unknown spot was detected, it was roughly estimated to 2 %. The following thin-layer chromatographic system according to *The International Pharmacopoeia*, Third Edition, Volume 4 was used.

Thin-layer:	Silica gel (Merck) HPTLC 60 F254.
Eluent:	7 % potassium dihydrogen phosphate.
Sample:	100 µg of streptomycin sulfate dissolved in water were applied.
Visualization:	Visualization in day-light after spraying with naphthalene-1,3-diol/ethanol and sulfuric acid.

$R_f(\text{streptomycin sulfate})=0.60$

$R_f(\text{unknown})=0.14$

$R_f(\text{kanamycin sulfate})=0.28$

$R_f(\text{neomycin sulfate})=0.09$

The detection limit of the system was about 0.5 µg (0.5 %) when examined visually after spraying.

The spot of streptomycin sulfate ICRS Control No 197215 corresponds in position and appearance with that of the EPCRS lot 1 of streptomycin sulfate.

High performance liquid chromatography

The total amount of impurities estimated by peak area normalization was about 6.0 % (n=5, RSD=0.08 % for the main peak, RSD=0.8 % calculated on the 2.6 % impurity level).

A chromatogram is shown in Figure 3. Five impurities above the limit of quantification were found. One of them was identified as dihydrostreptomycin (eluting at 20.5 minutes) by means of an external standard from National Institute for Biological Standards and Control (NIBSC 62/013) and estimated to 1.2 % (n=5, RSD=5.4 %). The main impurity was unknown 4, estimated to 2.6 % by peak area normalization. According to results from the validation of the method unknown 2, increased when stored in solution. It is here estimated to 1.3 % and might be stability indicating.

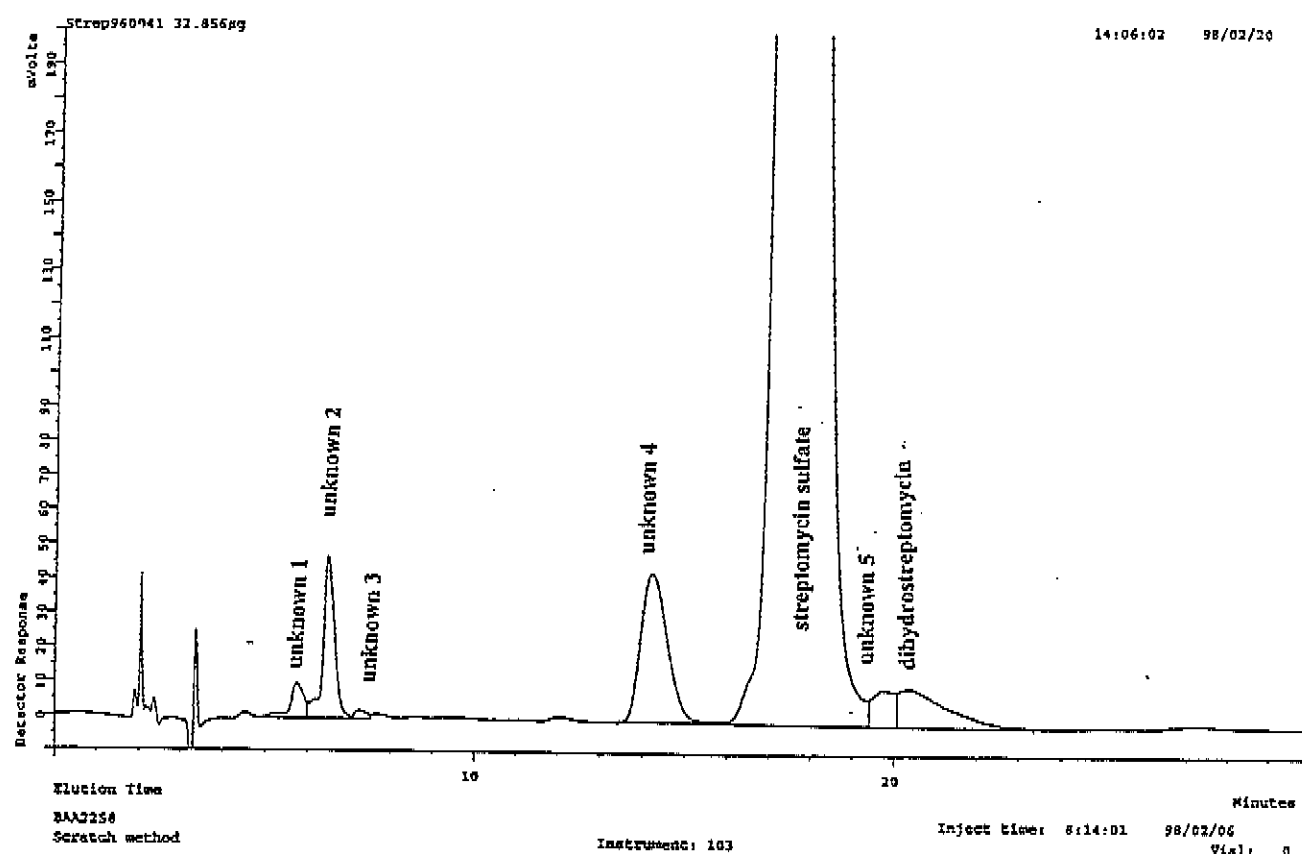


Figure 3. *Chromatogram of streptomycin sulfate Control No 197215 monitored at 200 nm.*

The following conditions were used:

Eluent:	0.1 M phosphate buffer at pH 3.8 was prepared. Then hexanesulfonic acid sodium salt was added to a concentration of 0.015 M. 100 ml acetonitrile were mixed manually with 900 ml buffer. (The low wavelength gives noise if the eluent is not properly mixed. The amount of organic solvent has strong effect on the retention time and resolution).
Column:	Kromasil C18, 4.6 x 250 mm, 5 μ m particles.
Detector:	Gynkotek DAD340S operated at 200 nm.
Pump:	Gynkotek 480 operated at a flow rate of 1.0 ml/min.
Injector:	Gynkotek Gina 50 operated at 8 $^{\circ}$ C
Integrator:	PeakPro (Beckman)
Sample:	Streptomycin sulfate was dissolved in water at a concentration of 2.0 mg/ml. 20 μ l corresponding to 40 μ g were injected.
Stability in the eluent:	A sample was stored for 1 week at room temperature. No signs of degradation were observed.
Limit of detection:	50 ng (0.1 %) at 200 nm
Limit of quantification:	100 ng (0.25 %) at 200 nm

Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of the impurity peaks as well as the main peak were similar. None of them had a distinct maximum but they all showed a slope increasing towards lower wavelengths. The wavelength of 200 nm was chosen, giving the best sensitivity for all peaks. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

Capillary Electrophoresis

Three impurities above the limit of quantification were found. Their total amount was estimated to be 5.2 % by peak area normalization ($n=13$, $RSD=0.5\%$ for the main peak, $RSD=13.6\%$ at the 2 % impurity level). Dihydrostreptomycin does not separate from streptomycin in this method, it is determined by liquid chromatography, see Purity/High performance liquid chromatography. An electropherogram is given in Figure 4.

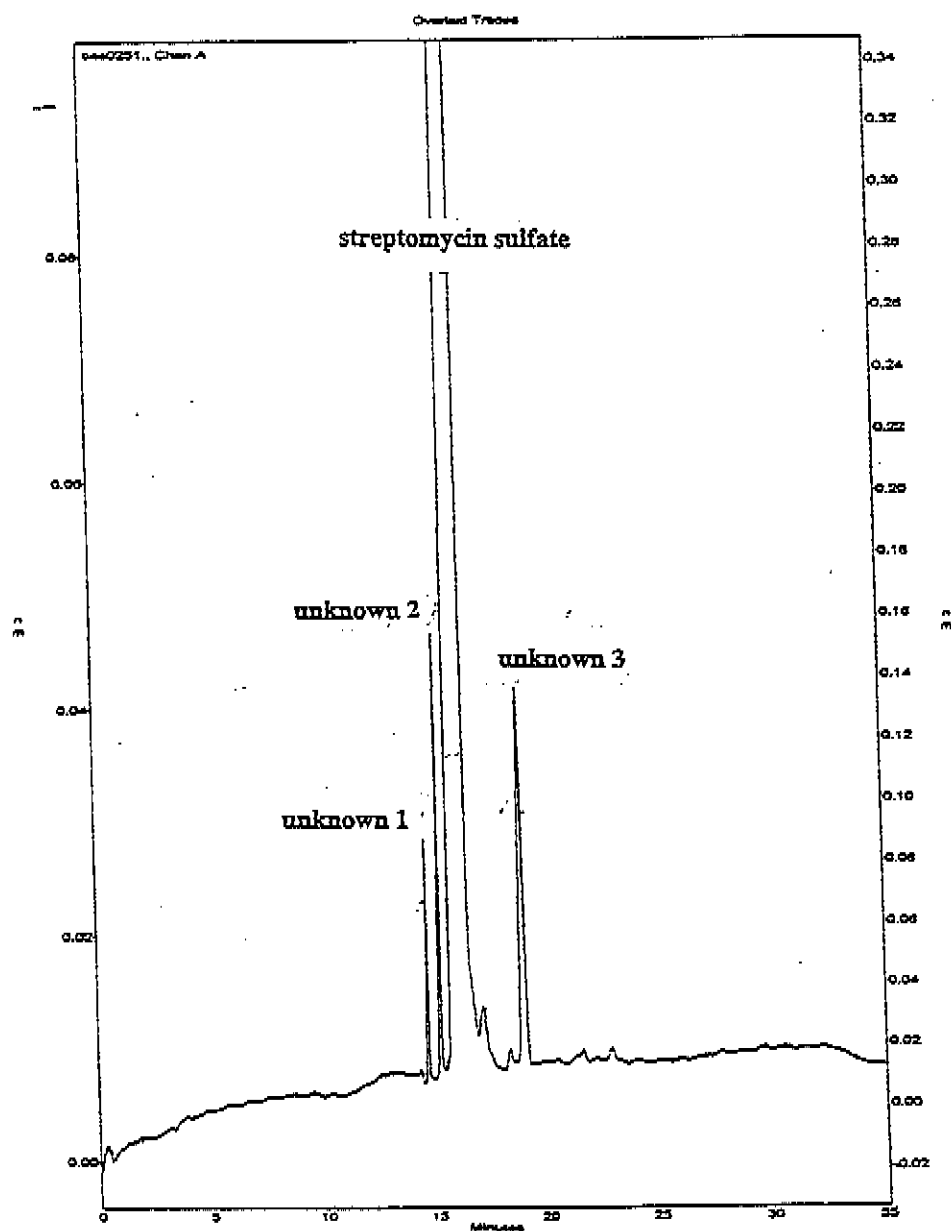


Figure 4. Electropherogram of streptomycin sulfate Control No 197215 monitored at 200 nm.

The following conditions were used:

Eluent:	100 mM phosphoric acid and 50 mM triethylamine, pH adjusted to 3.00 with 2 M sodium hydroxide
Rinsing of capillary:	3.0 minutes with 0.1 M sodium hydroxide 3.0 minutes with water 3.0 minutes with running buffer
Capillary:	Fused silica (total length 100 cm, length to detector 90 cm, ID: 75 μ m)
CE Instrument:	Beckman, P/ACE System MDQ operated with an applied voltage of 20 kV for 45 minutes.
Detector:	Beckman P/ACE System MDQ Diode Array Detector operated at 200 nm (bandwidth 10 nm).
Run temperature:	50 °C
Sample temperature:	8 °C
Injection:	20 seconds pressure injection, at a pressure of 0.5 psi.
Integrator:	Beckman P/ACE System MDQ soft ware
Sample:	Streptomycin sulfate was dissolved in water at a concentration of 3 mg/ml.
Stability in the eluent:	A sample was stored for one week at 8 °C in darkness. No signs of degradation were observed.
Limit of detection:	0.1 % at 200 nm.
Limit of quantification:	0.25 % at 200 nm.

Ion chromatography

The ICRS was screened for the following anions: chlorides, bromides, nitrates, phosphates and sulfates. Less than 10 ppm of bromides, less than 100 ppm of nitrates and less than 500 ppm of phosphates were found. Chlorides were found and estimated to 300 ppm. Sulfates were found and roughly estimated to be 21.9 % (theoretical value 19.8 %).

The following conditions were used:

Eluent 1:	Sodium carbonate/sodium bicarbonate 1.8 mM/ 1.7 mM
Eluent 2:	Sodium bicarbonate (for the determination of chloride) 2.5 mM
Column:	Dionex HPIC AS4A
Suppressor:	ASRS-1
Detector:	Dionex 2000i, conductivity
Range:	3 μ S (for determination of sulfate 300 μ S)
Pump:	Dionex 2000i
Injector:	Beckman System Gold Autoinjector 507
Integrator:	PeakPro (Beckman)

Sample: Streptomycin sulfate was dissolved in the eluent at a concentration of 1.0 mg/ml in water. 50 µl corresponding to 50 µg were injected.

Ion	Chlorides	Bromides	Nitrates	Phosphates	Sulfates
Limit of detection, ppm in the sample:	10	10	30	100	30
Limit of quantification, ppm in the sample:	50	50	100	500	100

Data given by collaborating laboratories

NIBSC

Description: The third International standard for Streptomycin established 1979.

Microbiological assay, determined in a collaborative study: 78500 International Units per ampoule.

Stability

Stability studies were not performed as this substance was not suspected to degrade easily when stored at -20 °C. Regular re-examinations of the ICRS when stored in the dry state will be performed.

Hygroscopicity

As the substance is very hygroscopic it is advisable to let it equilibrate with the surrounding atmosphere overnight if accurate weighing is requested.

Conclusion

Streptomycin sulfate, Control No 197215, can be considered suitable as International Chemical Reference Substance for the intended purpose. As this is a biological substance it is not possible to obtain a better quality.

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